Synthesis and Biological Evaluation of 4′-Hydroxymethyl Deleted, Transposed and Modified Nucleosides

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I thank all those, who contributed in their own way during these years of my life.

LIST OF ABBREVIATIONS

Ac	Acetyl
AIBN	Azobisisobutyronitrile
AIDS	Acquired immunodeficiency syndrome
AN	Apionucleoside
AR	Adenosine receptor
AZT	3′-Azidothymidine
BAIB	[Bis-(acetoxy)-iodo]benzene
BOM	Benzyloxymethyl
Bn	Benzyl
BuLi	*n*-Butyl lithium
BVDU	5-(*E*)-Bromovinyl deoxyuridine
CAN	Ceric ammonium nitrate
CDI	1,1′-Carbonyldiimidazole
CMV	Cytomegalovirus
D (Asp)	Aspartic acid
DCM	Dichloromethane
ddAN	Dideoxy apionucleoside
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azodicarboxylate
DMAP	4-Dimethylaminopyridine
Dm dNK	*Drosofila melanogaster* deoxyribonucleoside kinase
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DNApol	DNA polymerase
dTMP	Deoxythymidine monophosphate
dTDP	Deoxythymidine diphosphate
dUMP	Deoxyuridine monophosphate
E (Glu)	Glutamic acid
EC_{50}	Half-maximal effective concentration
EMCV	Encephalomyocarditis virus
ESI-HRMS	Electrospray ionization-HRMS
FDA	Food and drug administration
GPCR	G-protein coupled receptor
H (His)	Histidine
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCMV	Human cytomegalo virus
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography

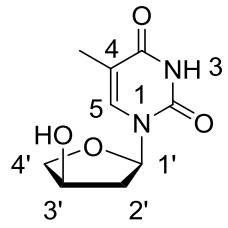
HSQC	heteronuclear single quantum coherence
HRMS	High resolution mass spectrometry
HSV	Herpes simplex virus
I (Ile)	Isoleucine
IB-MECA	N^6-(3-Iodobenzyl)-5′-(*N*-ethyl)-carboxamide adenosine
IC_{50}	Half maximal inhibitory concentration
K (Lys)	Lysine
L (Leu)	Leucine
LCMS	Liquid chromatography-mass spectrometry
m-CPBA	*meta*-Chloroperbenzoic acid
MW	Microwave
N (Asn)	Asparagine
NBS	*N*-bromosuccinimide
NDP	Nucleoside diphosphate
NDPK	Nucleoside diphosphate kinase
NECA	5′-*N*-ethylcarboxamide adenosine
NMI	*N*-methylimidazole
NMP	Nucleoside monophosphate
NMPK	Nucleoside monophosphate kinase
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
NP-DP	Nucleoside phosphonate diphsophate
NP-MP	Nucleoside phosphonate monophsophate
NTP	Nucleoside triphosphate
PBM cells	Peripheral blood mononuclear cell
PDB	Protein data bank
Phe (F)	Phenylalanine
Piv	Pyvaloyl
PMB	*para*-Methoxybenzyl
PMDT	3′-Phosphonomethoxy-2′-deoxythreosyl
ppm	Parts per million
p-TSA	*para*-Toluenesulfonic acid
Q (Gln)	Glutamine
QSAR	Quantitative structure-activity relationship
R (Arg)	Arginine
RNA	Ribonucleic acid
RNApol	RNA polymerase
RSV	Respiratory syncytial virus
RT	Reverse transcriptase
rt	Room temperature
SAH	S-adenosyl homocystine
SAR	Structure-activity relationship
SARS	Severe acute respiratory syndrome
SEM	2-(Trimethylsilyl)ethoxymethyl
S (Ser)	Serine

TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBDMS	*tert*-Butyldimethylsily
TEA	Triethylamine
TEAB	Triethylammonium bicarbonate
TEMPO	(2,2,6,6-Tetramethyl-piperidin-1-yl)oxyl
TCA	Trichloroacetic acid
THF	Tetrahydrofuran
T (Thr)	Threonine
TLC	Thin layer chromatography
TK	Thymidine kinase
TMPK*mt*	Thymidine monophosphate kinase of *M. Tuberculosis*
TMPK*h*	Human thymidine monophosphate kinase
TMS	Tetramethylsilane
TMSCl	Trimethylsilyl chloride
$TMSN_3$	Trimethylsilyl azide
TMSOTf	Trimethysilyl trifluoromethanesulfonate
TNA	Threose nucleic acid
UV	Ultraviolet
VSV	Vesicular stomatitis virus
VV	Vaccinia virus
VZV	Varicella zoster virus
Y (Tyr)	Tyrosine

NOMENCLATURE

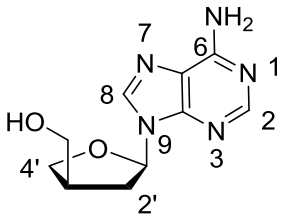
IUPAC nomenclature does not allow rapid visualization of carbohydrate and nucleoside structures. Hence, in this thesis compound names are derived from the parent sugar molecule and nucleobase, the use of which is accepted in this field of study (Figure 1). For instance the IUPAC name for compound **3.25** would be 1-((2R,4R)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione, while we prefer to denote it as 1′-(thymin-1-yl)-2′-deoxy-α-L-threofuranose.

However, in Chapter 6, which comprises nucleosides with many different functionalities and protecting groups, IUPAC nomenclature proved more practical.



3.25

1'-(Thymin-1-yl)-2'-deoxy-α-L-threofuranose

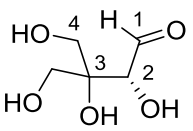


4.1b

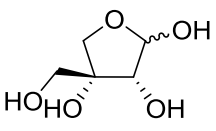
1'-(Adenin-9-yl)-2',3'-dideoxy-β-D-apio-D-furanose
OR
2',3'-dideoxy-β-D-apio-D-furanoadenosine

Figure 1

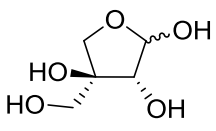
Apiose is a C-branched natural carbohydrate that has one asymmetric center in the open form but has an extra asymmetric carbon at position 3 in the cyclic furanose form (in addition to the anomeric center) (Figure 2).



D-apiose



D-apio-D-furanose



D-apio-L-furanose

Figure 2

Unconventially, the nomenclature of the cyclic forms of apiose therefore requires the use of two chirality designations (D, L) to distinguish between the two possible C3-

epimers. Alternatively, the prefixes *threo* and *erythro* may be used. D-Apio-D-furanose then corresponds with 3-*C*-(hydroxymethyl)-D-erythrofuranose. In literature, the natural D-apio-D-furanose is referred to as simply 'D-apiose'. In this manuscript the terms 'D/L-furano' is used to unambiguously specify the configuration at C-3.

PREFACE

The chemical modification of nucleosides has been and will continue to remain a valuable strategy towards antiviral and anticancer drugs. Also other applications of nucleoside analogues, for instance to modulate purinergic G-protein coupled receptors (*e.g.*, A_3 adenosine receptor) prove promising with several compounds under clinical investigation. Nucleoside analogues are also being developed as inhibitors of enzymes that play a crucial role in bacteria responsible for emerging infectious diseases (*e.g.*, thymidylate kinase of *M. tuberculosis*).

Nucleosides and especially their phosphorylated analogues are polar chemical entities, which hampers passive diffusion into the cells. Prodrug strategies may overcome this problem. In antiviral research, phosphoramidates are dual purpose prodrugs, which not only enhance the bio-availability but also bypass the first phosphorylation step (Figure 1).

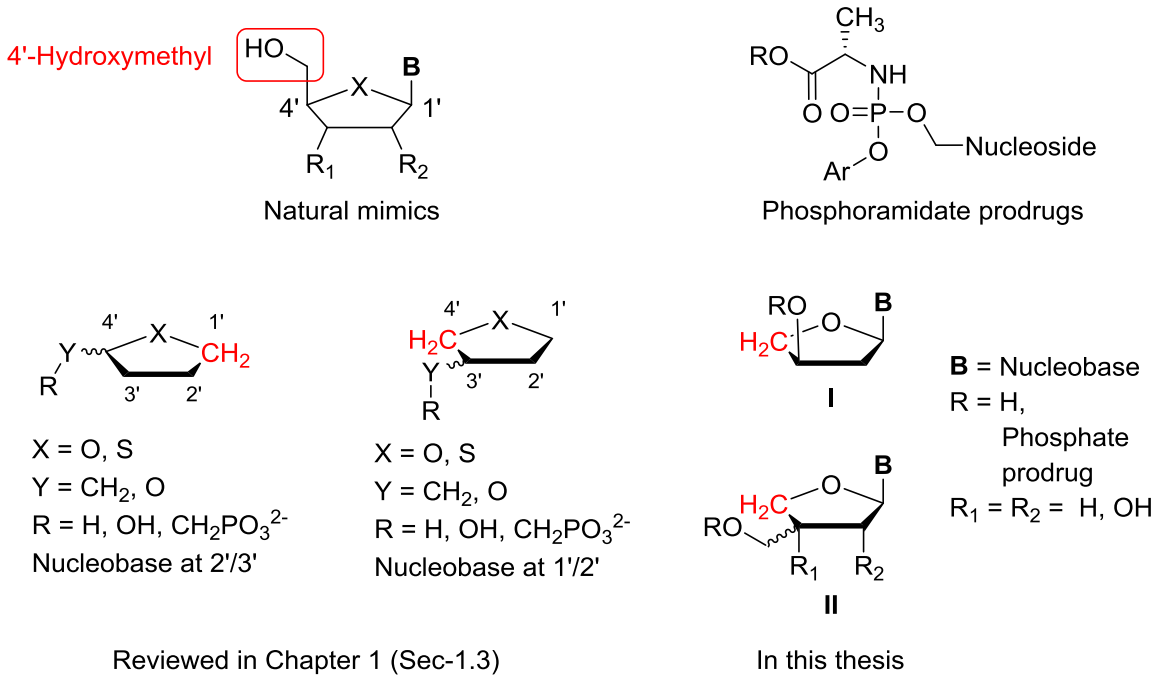


Figure 1

Nucleoside and nucleotide mimics, including acyclic analogues are successful as antivirals. The literature precedents and recent advances in the 1′/4′-CH_2 type

nucleosides were pivotal to the inception of this thesis, in which we will focus on 4′-hydroxymethyl deleted and transposed nucleosides (Figure 1). The rationale to undertake this work is described in Chapter 2.

Chapter 3 will describe the synthesis and biological evaluation of analogues **I**, while that of D-apio nucleosides **II** will be discussed in Chapter 4.

The new synthetic route developed to gain access to nucleosides **II**, will be employed to synthesize unprecedented apionucleosides as A_3 adenosine receptor ligands (Chapter 5).

Our group has reported some of the most potent inhibitors of this thymidine monophosphate kinase of *M. tuberculosis* (TMPK*mt*) (Chapter 1, sec 1.5). From an *in silico* quantitative structure activity relationship (QSAR) analysis, compound **IIIa** emerged as promising TMPK*mt* inhibitor (Figure 2). This led us to synthesized **IIIb**,**c** to test the effect of 5′ and 5-CH_2OH modifications on inhibitory potency (Chapter 6).

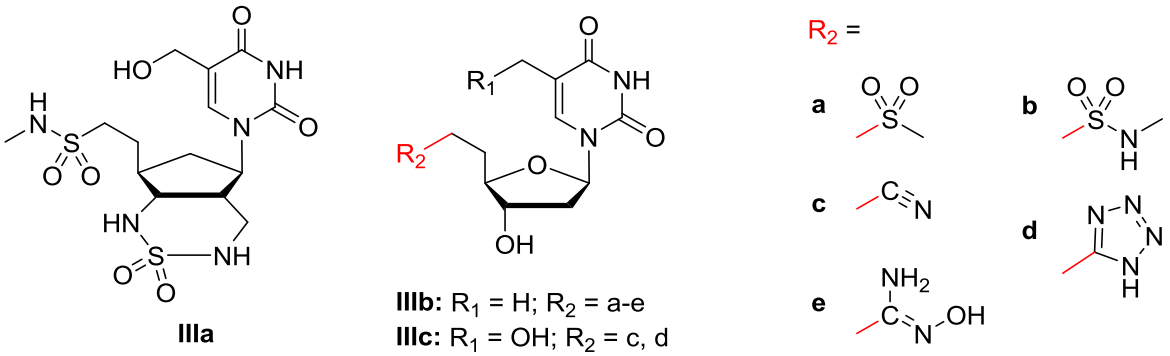


Figure 2

CHAPTER – 1

INTRODUCTION

1.1. Definition and Structure of Nucleosides

Nucleosides consist of a heterocycle (often called a nucleobase) attached to a sugar moiety through a glycosidic bond (Figure 1.1). When a nucleoside contains a phosphate residue at any oxygen of the sugar, it is termed a nucleotide. Many types of carbohydrates and bases exist but endogenous nucleosides generally contain one of the five bases bound to a D-ribofuranose or D-2′-deoxyribofuranose sugar to form RNA and DNA building blocks, respectively. These molecules are synthesized via two pathways. In the *de novo* pathway nucleosides are built up from basic small molecules. Purine nucleosides are constructed from activated carbohydrates, amino acids, N^{10}-formyl-THF (as a formate source) and carbonic acid. Pyrimidines are made from carbamoyl phosphate, amino acids, activated carbohydrate and N^{10}-formyl-THF (as 5-methyl donor). The major difference between the purine and pyrimidine pathways is that the former are built on the sugar moiety, while the pyrimidine core structure is built first and later attached to sugar, to form different pyrimidine nucleosides. In the *salvage* pathway, nucleosides are recovered from degradation products. Comparatively, the latter is less energy demanding. Nucleosides are the basis of genetic material, play an important role in signaling pathways and are also involved in energy transfer and storage.

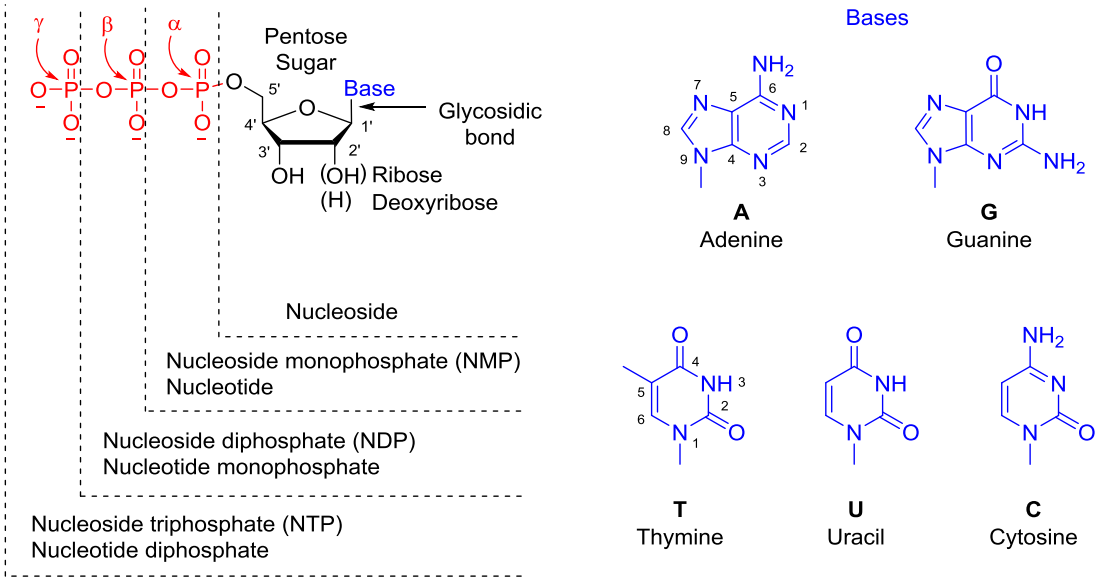


Figure 1.1. Structure of nucleos(t)ides

1.2. Conformations of the Nucleoside

The conformation of a nucleoside can be defined by three parameters: the glycosyl torsion angle (χ), the torsion angle (γ) between 5′-OH and 3′-C, and the furanose ring puckering, known as pseudorotation (*P*). The structural change in nucleosides caused by pseudorotation is highlighted in Figure 1.2.[1] In solution nucleosides are in dynamic equilibrium between Northern (N, 3′-endo-2′-exo, ${}_2E$, ${}^3T_2$, ${}^3E$) and Southern (S, 3′-exo-2′-endo, ${}^2E$, ${}^2T_3$, ${}_3E$) type conformations but often preferentially adopt one of these two conformations. In addition, the orientation of the nucleobase (*syn/anti*) and 5′-hydroxyl group influence the overall conformation in solution. Notably, the conformation in solid state (crystals) may be different from the conformation in solution. Even though the energy difference between conformations is relatively small, enzymes may have outspoken preferences for typical nucleoside conformers and conformationally non-adaptive molecules may be differently recognized by enzymes in a given biological sequence.

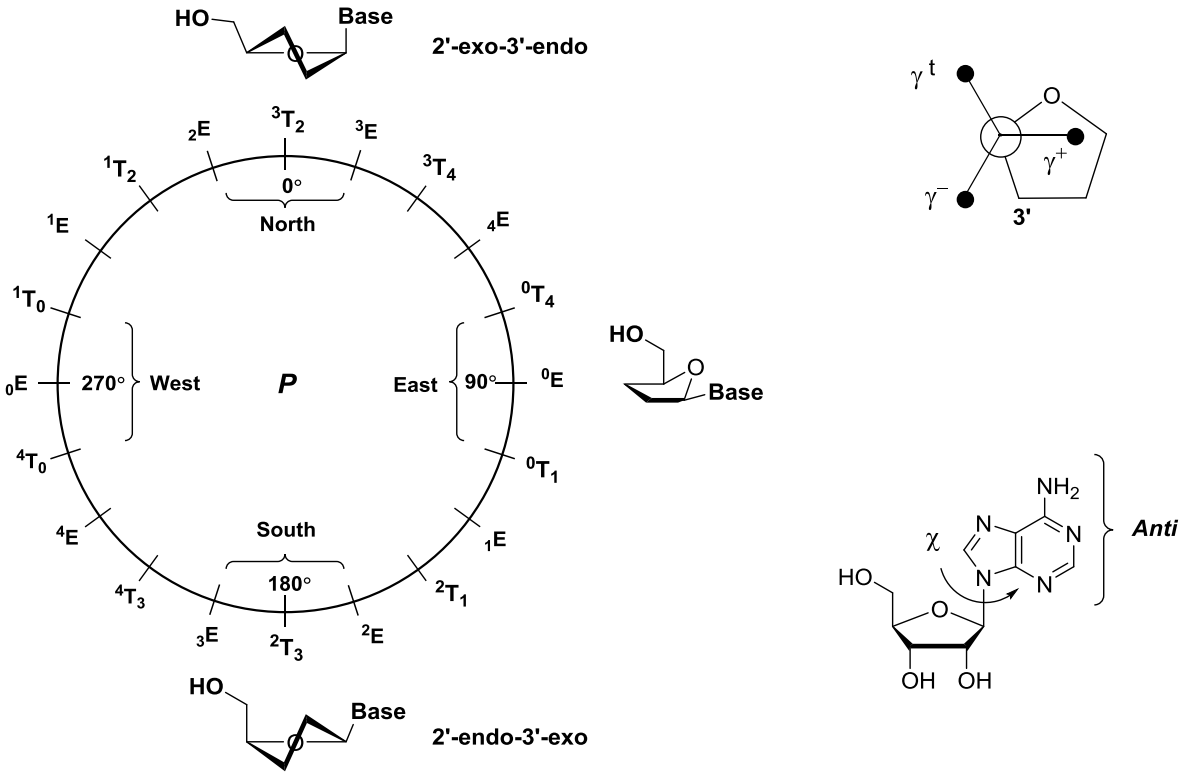


Figure 1.2. Pseudorotational cycle of nucleosides (left), torsion angle γ (top right) and χ (bottom right).

The crucial importance of the conformation or conformational flexibility for the biological activity of nucleosides was nicely demonstrated by Marquez *et al.* using nucleoside analogues that are locked in one conformation.[2] From these and other studies[3] it became clear that nucleoside kinases readily phosphorylate S-conformers, while HIV-1 RT preferably binds nucleoside triphosphates (such as AZT-TP) in an N-type conformation.[4] The unambiguous proof comes from the study of locked nucleosides. The triphosphates of bicyclic nucleoside mimics (N)-*methano*-carba-AZT (Figure 1.3, **1.1**) and (S)-*methano*-carba-AZT (**1.2**) were investigated as inhibitors for HIV RT (recombinant and wild type), showing that inhibition of RT occurred only with the conformationally locked (N)-*methano*-carba-AZT 5′-triphosphate. The inhibition was equipotent to and kinetically indistinguishable from that produced by AZT 5′-triphosphate. The 5′-triphosphate of (S)-*methano*-carba-AZT, by contrast, did not inhibit RT.

1.1: $_2E$, N-Type **1.2: $_3E$, S-Type**

Figure 1.3. Conformation of locked nucleosides *methano*-carba-AZT.

1.3. Nucleosides as Antivirals[5]

Modified nucleosides were first used in cancer chemotherapy and are still being investigated for therapeutic use in oncology (*e.g.*, citarabine, one of the nucleoside still in use was approved by the FDA in 1969). The use of nucleosides as antivirals became widespread only after the discovery of the human immunodeficiency virus (HIV) and which fueled antiviral drug development.[6] 3′-Azidothymidine (AZT), whose synthesis was reported already in 1964,[7] was the first drug licensed for HIV treatment, thereby becoming the first approved nucleoside analogue for antiviral

therapy.[8] Even after three decades of research, treatment of chronic infections caused by HIV, HCV, HBV and acute infections (*e.g.* influenza, SARS, etc.) is proving difficult. In the absence of effective vaccines against these viruses, common problems in antiviral chemotherapy involve toxicity, adverse effects, drug-drug interactions, emergence of new viruses and, last but not least, the development of drug resistance.

1.3.1. Mechanism of action of nucleosides/ nucleoside phosphonates as antivirals

In a strict sense most nucleoside drugs are prodrugs. They are metabolized by cellular or viral kinases to their corresponding 5′-nucleoside triphosphates (NTP), which are the actual inhibitors of DNA or RNA polymerases (Figure 1.4). Structural requirements for antiviral activity include:

- Sterically non-crowded hydroxyl group for phosphorylation (so far only primary hydroxyls have been demonstrated to be effectively triphosporylated) and a nucleobase (mimic).
- Orientation of the nucleobase and the primary hydroxyl group on the same side of the ring.
- Ring systems should be sufficiently flexible to adapt the conflicting conformations for kinase activation and polymerase binding.

A nucleoside first undergoes phosphorylation by nucleoside kinases to its 5′-monophosphate (NMP). Very often, this is the rate-determining step in the activation process (AZT, is a well-known exception[9]). Nucleoside phosphonates have been developed to bypass this first phosphorylation step. The NMP is converted to the NDP (or nucleoside phosphonate to NP-MP) by the action of NMPKs. Subsequent phosphorylation by NDPKs yields the NTP (or NP-DP). The triphosphate analogue interacts with viral DNA/RNA polymerase either as a competitive inhibitor or as a substrate. Since antiviral nucleosides often lack a 3′-hydroxyl group, their incorporation eventually leads to DNA/RNA chain termination.

Due to the close structural similarity of the triphosphorylated antiviral nucleosides and the natural NTPs, the former may also interact with host cell DNA/RNA polymerases

resulting in toxicity. This is the main cause for mitochondrial toxicity. The toxicity may be diminished to a large extent in the case the virus produces its own kinases, making it possible to design viral kinase/ polymerase specific nucleosides. Non-natural L-nucleoside analogues have also proven successful in lowering mitochondrial toxicity due to differential uptake by mitochondrial transporter proteins.

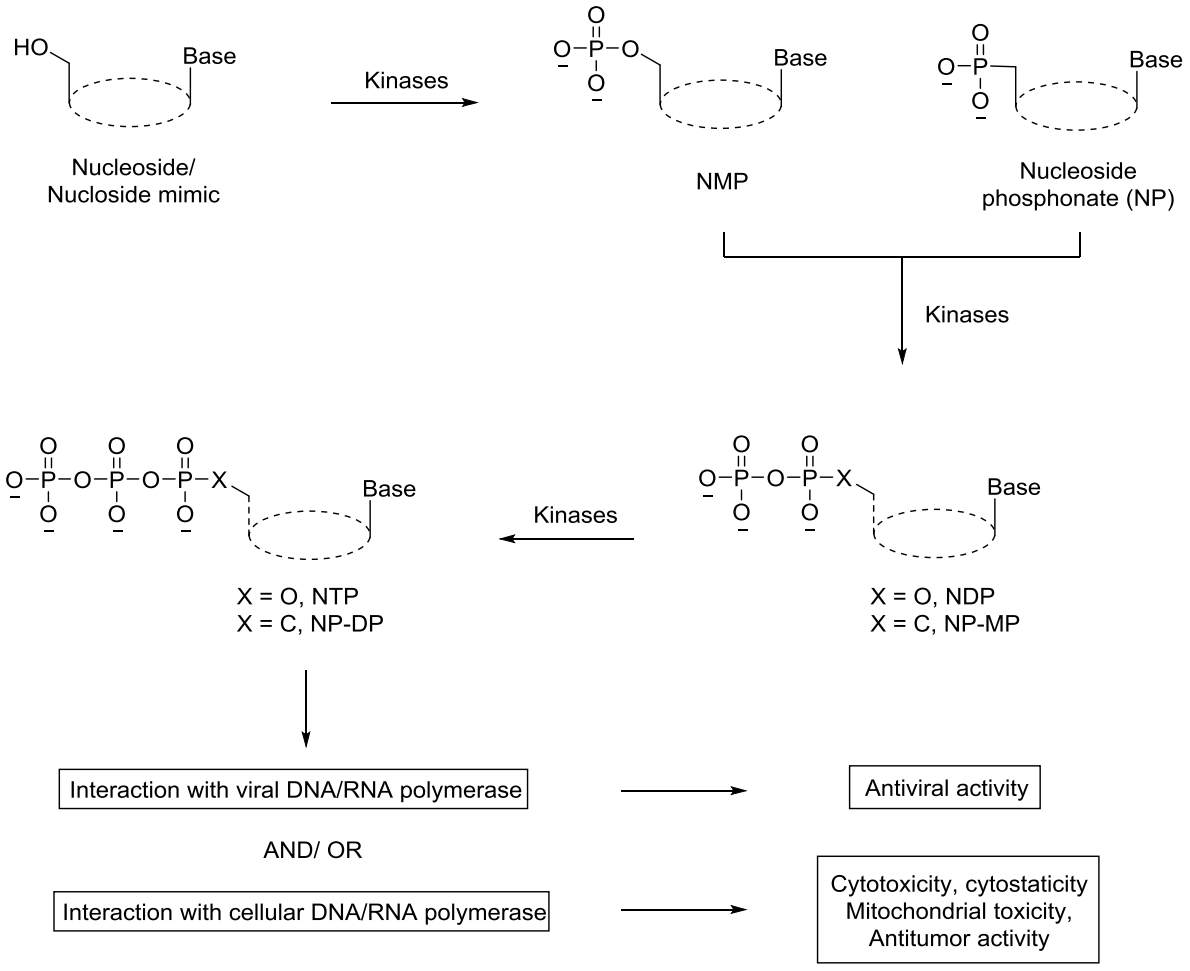


Figure 1.4. General mode of action of nucleosides/ phosphonates.

In recent years, nucleoside analogs have also been found useful to inhibit crucial enzymes such as S-adenosyl homocystine (SAH) hydrolase, viral/cellular RNA helicase and inosine monophosphate dehydrogenase.

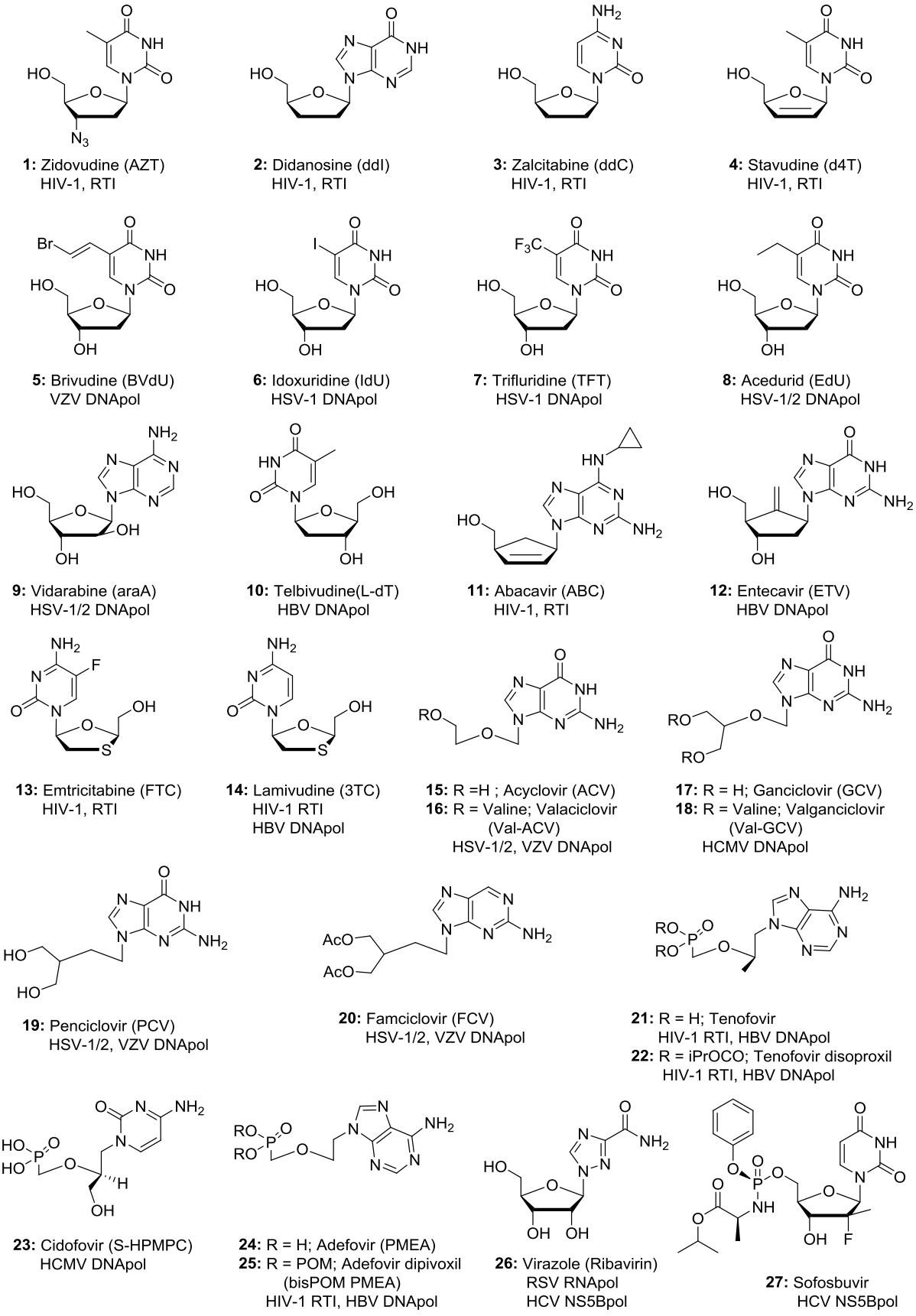
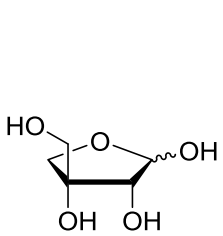


Figure 1.5. Nucleoside/ nucleotide analogs approved for therapeutic use.

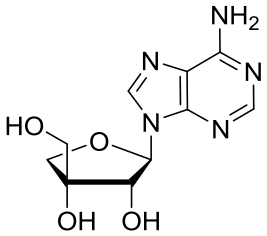
At present, there are 25 approved nucleoside/nucleotide analogs in use as antivirals (Figure 1.5). Generally, these nucleos(t)ides closely resemble natural nucleosides and are characterized by slightly modified bases, non-natural L-sugars, carbocyclic rings, bis-hetero-sugar moieties, acyclic nucleosides and nucleoside phosphonates. The only drug featuring with an intensively modified base moiety is ribavirin (Figure 1.5, **26**).

1.3.2. The 4′-hydroxymethyl/ base transposed nucleosides/ nucleoside phosphonates

1.3.2.1. Apionucleosides



1.3



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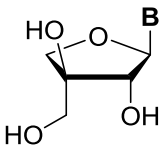
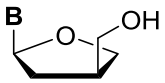


1.5a: A
1.5b: T
1.5c: U
1.5d: 5Br-U
1.5e: 5I-U

D-apio-D-furanose (**1.3**) is a branched natural carbohydrate. Apiose derivatives, including nucleosides such as β-D-apio-D-furanoadenosine (**1.4**) are mostly found in plants.[10] The presence of the D-apio-D-furanose form in all naturally occurring apiosides is explained by the fact that the transferases catalyze the transfer of the *erythro*- but not the *threo*-furanose from UDP-D-apiose to the various acceptors.[11] The first syntheses of apionucleosides were reported by Riest *et al.*[12] and Carey *et al.*[13] Detailed studies on the chemistry, physical properties and evaluation of apionucleosides were carried out in the laboratory of J. Tronchet. It was demonstrated that the apionucleosides exist mainly in the $E_{3'}$ (S-type) conformation and that β-D-apio-D-furanoadenosine nucleoside **1.4** is resistant to adenosine deaminase, while its α-L-furano counterpart **1.5a** is a substrate.[14] Medicinal chemistry aspects of these molecules were first explored by Watson *et al.* who evaluated them against T-lymphocyte proliferation and HSV.[15] Compound **1.5a** significantly reduced HSV-2

plaque formation in BHK cells (42% reduction at 4 µM), while **1.4** did not and all the α-L-nucleosides (**1.5a-e**) inhibited rat T-lymphocytes/ human MGL8 lymphoblasts.

Initially, the adenine analogue of **1.6** (L-ddAA) was reported to show weak anti-HIV activity in MT-4 cells (data not provided)[16], but subsequently it is found that series **1.6**, **1.7** and **1.8** are neither cytotoxic nor active.[17] The homologated dideoxyapioadenosine **1.9** and its enantiomer were found inactive too, although they were shown to be resistant to adenosine deaminase and to be hydrolytically more stable than ddA.[18]



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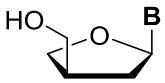

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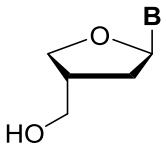

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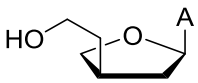
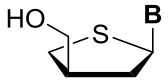
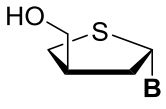


1.9

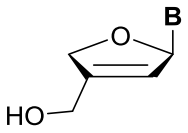
The bioisosteric thioapio dideoxynucleosides **1.10** and their anomers **1.11** (both tested as enantiomeric mixtures) were tested against HIV-1, but did not afford any promising compounds due to cytotoxicity. In particular the 6-Cl-purines **1.10** and **1.11** showed cytotoxicity in MT4-cells (CC_{50} <8.5 and <5 µM respectively). This prompted the authors to assess the potential of 6-Cl-purine **1.11** in colon-2 cancer cells (IC_{50}: 10.3 µM).[19] Most apio-dideoxydidehydro nucleosides **1.12** showed moderate to potent anti-HCMV activity without cytotoxicity, the 5-fluorouracil derivative being the most potent (EC_{50}: 30.2 µM in AD-169 cells and 12.3 µM in Davis cells). However, the corresponding thioapio analogues did not exhibit any significant anti-HCMV activities.[20]



(±)-1.10: B = U, T, A, G, 6-Cl-purine, N^6-MeA Inosine



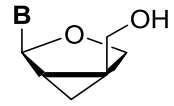
(±)-1.11: B = U, T, A, G, 6-Cl-purine, N^6-MeA Inosine



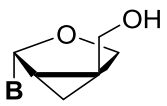
(±)-1.12: B = T, U 5-F, Cl, Br, I-U

A series of N-type apionucleosides was synthesized, which are somewhat related to (N) methanocarba-T (**1.15**), a potent anti-HSV agent. **1.13**T demonstrated toxicity

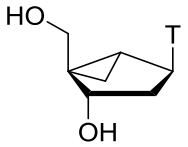
dependent anti-HIV-1/2 activity (EC_{50}: 190.6 µM).[21] 2′,3′-Cyclopropyl fused α-nucleosides **1.16** were devoid of antitumor activity, but no antiviral data have been reported.[22]



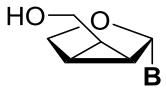
1.13: B = A, T
C, U



1.14: B = A, T
C, U

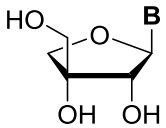


1.15

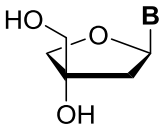


1.16: B = T, A
G, guanin-7-yl

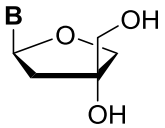
The pyrimidine apionucleosides **1.17** and their corresponding 2′-deoxy congeners **1.18** were reported to be inactive against a panel of viruses (HIV-1/2, HSV-1/2, VV, VSV, etc), while the later series **1.18** was found slightly toxic for Vero cells. The enantiomeric 2′-deoxy compounds **1.19** and anomers **1.20** were also found inactive.[23]



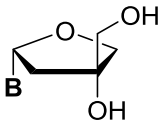
1.17: B = T, U, C



1.18: B = T, U, C

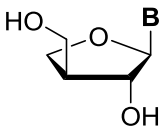


1.19: B = T, U, C

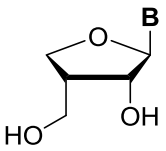


1.20: B = T, U, C

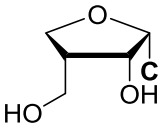
The D- and L-3′-deoxynucleosides **1.21** and **1.22** failed to inhibit DNA or RNA viruses (HIV-1/2, HSV-1/2, VV, VSV, etc.) and, unlike their 2′-deoxy regiomers **1.18**, showed no cytotoxicity.[24] The triphosphates of **1.21** and natural nucleic acids were found to form chimeric oligomers by many DNA polymerases.[25] The cytidine congener **1.23** was also shown to be inactive against HIV.[26] Jung *et al.* found 2′- azido and fluoro analogs **1.24** inactive against a panel of viruses, but found the thymidine analogue of **1.21** active against HSV-1(KOS) (MIC_{50}: 39.6 µM), and the guanine analogue active against HSV-1 in two different cell lines *i.e.* KOS and 2(G) (MIC_{50}: 7.1 and 35.9 µM, respectively).[27]



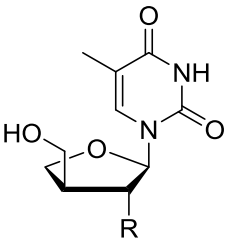
1.21: B = T, U, C
A, G



1.22: B = T, U, C

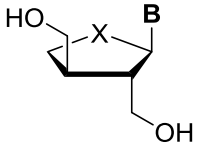


1.23

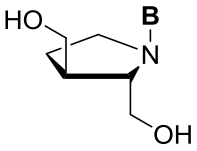


1.24: R = N_3, F

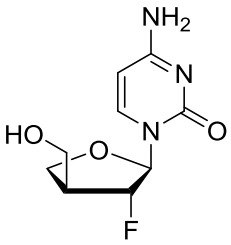
The ring expanded oxetanocin analogues **1.25**, **1.26** and an unusual azasugar nucleoside **1.27** were inactive against HIV-1, HSV-1/2.[28] Antiviral assays against HIV-1, HSV-1/2, HCMV involving the racemate of **1.28** and its anomer did not yield encouraging results.[29] No biological data have been reported for compounds **1.29** and their enantiomers.[30]



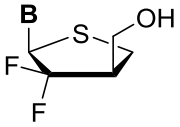
1.25: X = O; B = A, T, C
1.26: X = S; B = A, T



1.27: B = A, T, G

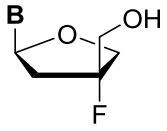


(±)-1.28

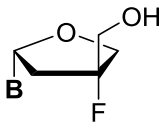


1.29: B = T, 5-F-U

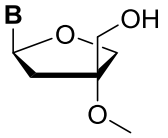
The 3′-F-2′,3′-dideoxyapionucleosides **1.30** and **1.31** are amongst the most promising in the apionucleoside class. These compounds are highly active against HBV. The 3′-methoxy analogs **1.32** and **1.33** are claimed equally effective against this virus. *In vitro* activity data are reported for selected compounds only (T, C, 5-F-C, and 5-I-U). Among these the cytosine analogue **1.30** is the most potent (EC_{50}: 0.011 µM) with a >10,000 fold selectivity index.[31] Notably, enantiomers of **1.30** and **1.31** (A and C analogs) do not show significant activity against HIV, HSV-1/2 and poliovirus, but their ability to inhibit HBV is not reported.[32]



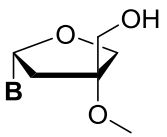
1.30: B = T, C, 5-F-C, 5-F-U, 5-Cl-U, 5-I-U, G, 6-Cl-purine



1.31: B = T, C, 5-F-C, 5-F-U, 5-Cl-U, 5-I-U, G, 6-Cl-purine

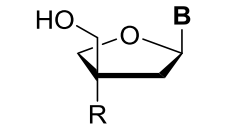


1.32: B = T, C

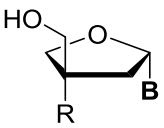


1.33: B = T, C

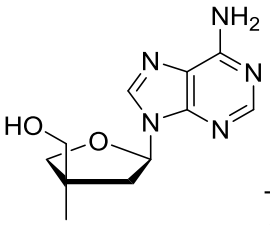
The racemic mixture of **1.35** (B = A) was found to be active against HBV (EC_{50}: 3.5 µM). The (*R*,*R*) isomer was synthesized stereoselectively and found inactive, hence it was inferred that the active compound is (*S*,*S*) isomer. No other analogues of this type showed antiviral activity against HBV, HIV-1, HSV-1/2, HCMV and poliovirus, nor cytotoxic activity.[33] Compound **1.38** showed moderate activity against HIV (EC_{50}: 2.55 µM). Interestingly, compound **1.39** showed much better activity, but also significant toxicity (EC_{50}: 0.073 µM, IC_{50}: 1.0 µM in PBM cells). However, none of these compounds showed any significant anti-HBV activity up to 100 mM.[34] Biological data for their 4′-thio congeners are not presented.[35]



(±)-1.35: R = NH_2, B = A, C
(±)-1.36: R = N_3, B = A, C

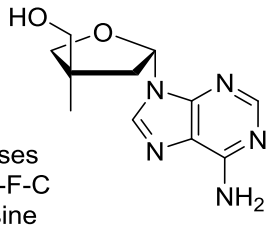


(±)-1.37: B = A, C; R = N_3, NH_2



1.38

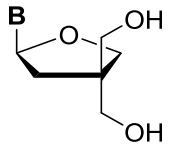
Other Bases
T, U, C, 5-F-C
A, G, Inosine



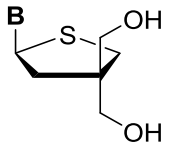
1.39

The 2′-deoxy-3′-hydroxymethyl apioside **1.40** demonstrated weak anti HIV-1 activity (EC_{50}: 75.8 µM), while its thymine congener is still weaker (EC_{50}: 199 µM). Upon replacement of the oxygen by sulphur, the adenine derivative lost its activity completely, while cytidine **1.41** gained activity against HIV-1 (EC_{50}: 74.7 µM) and HCMV (EC_{50}: 133.2 µM).[36] When the hydroxymethyl group *anti* to the base was replaced by a vinyl group, the resulting compound **1.42** (B = U) proved active against HIV-1 (EC_{50}: 72.6 µM) and HCMV (EC_{50}: 187.6 µM). Substituting the *syn* hydroxymethyl to a vinyl led to some activity loss (B = T; 238.6 µM, B = U; 331.2 µM).[37] Replacing the vinyl moiety in **1.42** by phenyl rendered the molecule inactive.[38] If we take into account the data from compounds **1.30** to **1.39**, it is evident that the 3′-

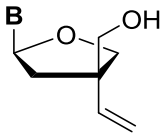
substitution is very important for activity, possibly by imposing a favorable conformation/ molecular orientation.



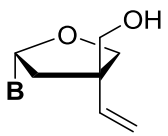
1.40: B = A (C, T, U)



1.41: B = C (A, T, U)

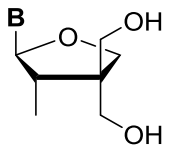


1.42: B = T, U

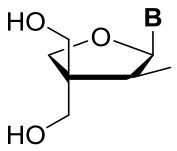


1.43: B = T, U

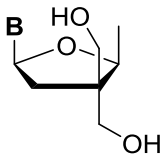
Compound **1.44**A was moderately potent against HIV-1 (EC_{50}: 36.1 µM) and also showed moderate HSV-1 (EC_{50}: 116.7 µM) and HCMV activity (EC_{50}: 148.9 µM). The antiviral activity of **1.45**A is 2-fold weaker compared to **1.44**A against all viruses. Amongst the pyrimidine analogues only analogues **1.44** showed anti-HCMV activity, **1.44**U and **1.42**U,T showed anti-HSV-1 activity, while **1.44**T,C showed anti-HSV-2 activity.[39] Compound **1.46**A inhibited the replication of HCV (IC_{50}: 19 µM).[40]



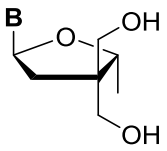
1.44: B = U, T, C, A,



1.45: B = U, T, C, A,



1.46: B = A, C

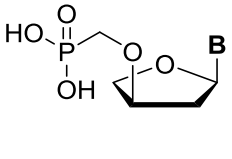


1.47: B = A, C

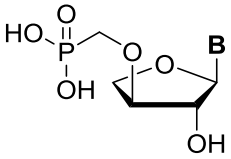
1.3.2.2. Apionucleoside phosphonates

Herdewijn *et al.* reported the synthesis and anti-HIV properties of the first apionucleoside monophosphate bioisosteres, namely the threosylphosphonate compound series **1.48**.[41] The adenine and thymine derivatives showed promising anti HIV-1/2 activity (EC_{50}: 2.36 µM and 6.59 µM, respectively) without significant toxicity (>320 µM). The diphosphate of phosphonomethoxydeoxythreosyladenine (PMDTA, **1.48**A) was incorporated by hDNApol-α only at enzyme concentration that are 100 times higher than those required for the dATP substrate. On the other hand HIV RT accepts this diphospophosphonate readily with only 2.5 times slower kinetics compared to dATP. This makes it a promising candidate for development as a

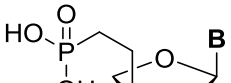
potential anti-HIV drug. Interestingly, substituting the 3′-oxygen with a methylene decreases the efficacy (**1.50**: EC_{50}: 12.6 µM), while increasing toxicity (30.4 µM in CEM cells).[42] Although, the 2′-hydroxy series **1.49** is inactive against HIV replication, these molecules are readily accepted by the therminator polymerase to form oligophosphonates.[43]



1.48: B = A, T
C, U,

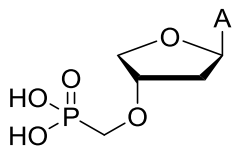


1.49: B = A, T
C, U,

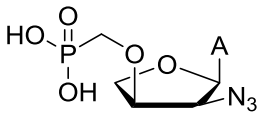


1.50: B = A, G

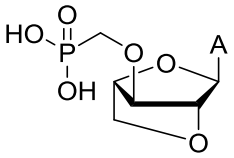
Further modifications to **1.48**A led to compounds **1.51**-**56**, which all were inactive against HIV. The triphosphate of the locked phosphonate **1.53** was ineffectively incorporated by HIV RT. Only at very high substrate and enzyme concentrations (400 µM and 1.44U/µL) 18% of the template was elongated.[44]



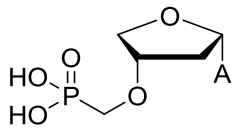
1.51



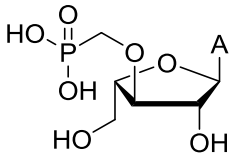
1.52



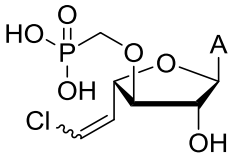
1.53



1.54

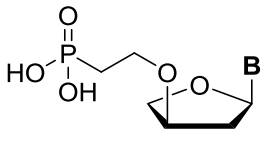


1.55

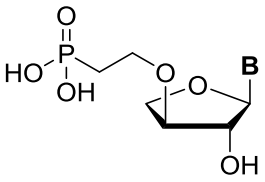


1.56

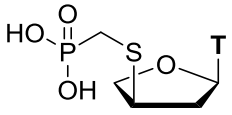
Recently, more modified PMDTA analogues were reported, such as the spacer elongated variants **1.57** and **1.58**, the 3′-thio congeners **1.59** and **1.60**, the unsaturated phosphonates **1.61** and **1.62**, the all carbon 3′- elongated **1.63** and the cyclic apiosyl phosphonate **1.64**.[45] None of these exhibited anti-HIV, HCV, RSV or cytotoxic properties.



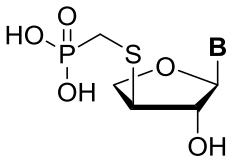
1.57: B = A, T



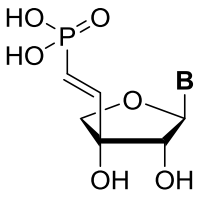
1.58: B = A, T



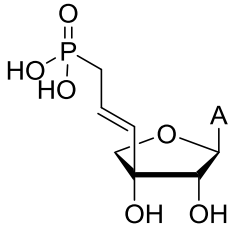
1.59



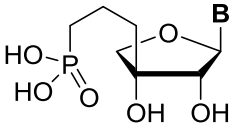
1.60: B = A, T



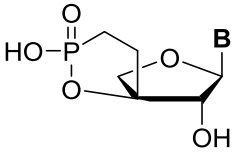
1.61: B = A, T



1.62

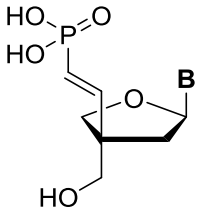


1.63: B = A, T

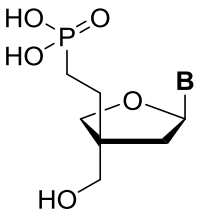


1.64: B = A, T

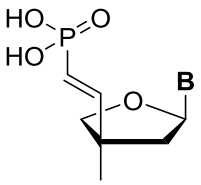
Similar to compound **1.50**, **1.66**G combined significant anti-HIV-1 activity (EC_{50}: 10.2 µM) with cellular cytotoxicity (IC_{50}: 45.5 µM in PMB, and 32.0 µM in CEM cells). Other phosphonates (**1.65** and **1.66**A) exhibited weak anti-HIV-1 activity (EC_{50}: 45-80 µM) without cytotoxicity (>100 µM). A similar trend was followed by **1.67** and **1.68**. **1.68**A was moderately active against HIV-1 (22.2 µM) with cytotoxicity in PMB (42.4 µM) and CEM cells (30.4 µM) and the EC_{50} of the other analogues was in the range 50-85 µM. A modelling study pointed towards a shift of the phosphonate moiety of **1.66**G compared to that in PMDTA, while **1.68**A also showed a slight shift of base.[46]



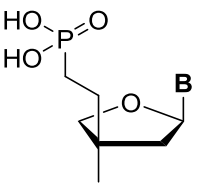
1.65: B = A, G



1.66: B = A, G



1.67: B = A, G

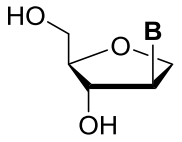


1.68: B = A, G

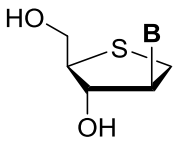
1.3.2.3. Isonucleosides

In an effort to discover nucleosides that can withstand chemical and enzymatic glycosidic cleavage, Montgomery and Thomas synthesized the first isonucleosides **1.69a** and **1.69b**.[47] These were found inactive against leukemic 1210 cells. Later

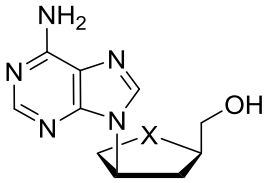
studies showed that compound **1.69a** demonstrates reasonable anti-HSV1/2 (EC_{50}: 78.8, 93.9 µM, respectively) and HCMV (EC_{50}: 76.4 µM) activity, in addition to toxicity in L1210 cells (IC_{50}: 71.6 µM). The guanine analogue **1.69d** proved four times more potent than **1.69a** against HSV1/2 (EC_{50}: 15.3 and 22.8 µM, respectively) without noticeable cytotoxicity, while other bases were inactive.[48] The 4′-thio congeners **1.70** were found inactive against all tested viruses (HIV, HSV-1/2, VZV, and HCMV) and demonstrated no cytotoxicity.[49]



1.69a: B = A ; **1.69b:** B = U
1.69c: B = C ; **1.69d:** B = G
1.69e: B = 2,6-diaminopurine



1.70: B = A, G,
2-aminoadenine
T, 5-F-U, 5-BV-U

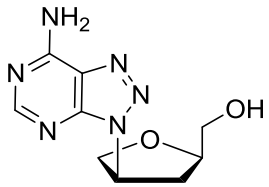


1.71: X = O
1.72: X = S

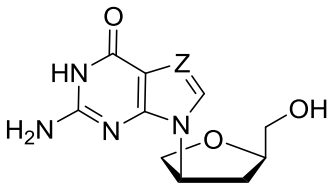
Ten years after the first synthesis of isonucleoside **1.69a**, its 3′-deoxy-enantiomer (*R*,*R*)-isoddA **1.71** was reported to possess potent anti-HIV activity (ED_{50}: 5-20 µM in different cells).[50] The guanine counterpart was less active than **1.71,** while other base variations did not show any anti-HIV activity. Compound **1.71** is highly resistant to adenosine deaminase and intracellular activation to its triphosphate occurs better than for the ddA isomer.[51] Phosphoramidate and SATE prodrugs of **1.71** increased the anti-HIV activity almost 400 fold.[52] In fact the ProTide of **1.71** is as effective as AZT (EC_{50}: 0.04 µM, MT-4 cells) against HIV-2. However this ProTide also possesses considerable toxicity with a selectivity index of only 65.[53] Compound **1.71** also displayed weak binding affinity to A_{2A} and A_3 receptors while it was found inactive at A_1 receptor.[54] The 4′-thio derivative **1.72** and other base analogues were inactive against HIV.[55]

Interestingly, the triphosphate of the 8-azaadenine congener **1.73** inhibits recombinant RT almost eight times more effectively (IC_{50}: 4.3 µM) than the triphosphate of **1.71** (IC_{50}: 35 µM), while **1.71** is much more potent in acutely infected cells. This anomaly could be due to a different uptake of the two compounds by infected cells.[53] The

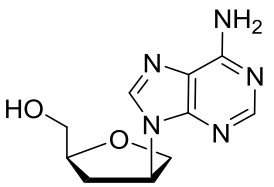
dideoxyisoguanine **1.74** was marginally active against HSV-1/2 (MIC: 8-16 µM) and virtually inactive against vaccinia virus and TK deficient mutants of HSV-1, a trend that is similar to that of acyclovir.[56]



1.73

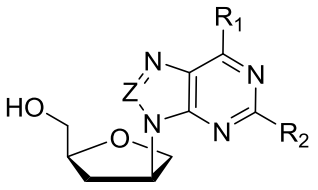


1.74: Z = N
1.75: Z = CH



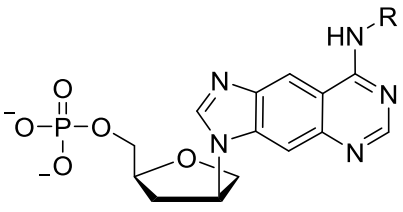
1.76

Nair and co-workers reported that (*S*,*S*)-isoddA (**1.76**) displayed twenty times better anti-HIV-1 (IC_{50}: 0.67 µM) activity than its enantiomer **1.71**.[57] The inhibition of HIV-1 RT by the triphosphate of **1.76** (K_i: 16 nM) is comparable to that caused by AZT-TP (K_i: 4 nM). IsoddATP and ddATP, exhibit complimentary results for human DNA polymerases. ddATP is a strong inhibitor of DNA polymerase β and γ (K_i: 1.1 and 0.018 µM, respectively), while isoddATP effectively inhibits DNA polymerase α (K_i: 0.63 µM). (*S*,*S*)-IsoddA is insensitive to deaminases and even acts as a weak inhibitor of this enzyme. However, isoddA was found to inhibit the growth of human bone marrow progenitor cells. Compound **1.76** also exhibited HBV activity *in vitro* (IC_{50}: 3.3 µM; CC_{50}: ~140 µM) but showed *in vivo* toxicity.[58] Compound **1.76** predominantly adopts a northern conformation in solution (58%), while it appeared in the southern conformation in the crystal structure.[59] In solution, the nucleosides with anti-HIV activity typically adapt the southern conformation.[60]

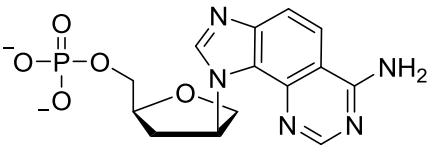


1.78

$R_1 = NH_2$, R_2 = OH, Z = CH
$R_1 = NH_2$, R_2 = H, Z = COH
$R_1 = NH_2$, R_2 = H, Z = N
R_1 = H, R_2 = H, Z = CH

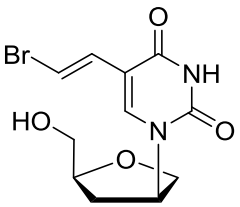


1.79: R = H
1.80: R = $(CH_2)_8NH_2$

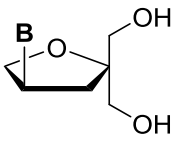


1.81

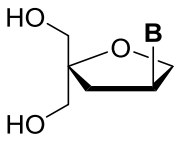
Stereochemical modifications at the 2′ and 4′-carbon and various base modifications, as in **1.78**, yielded inactive or marginally active compounds.[61] Isonucleosides with tricyclic bases caused inhibition of HIV integrase, a viral enzyme responsible for incorporating viral double stranded DNA in to host chromosomal DNA genome. The integrase enzyme recognizes the specific sequence (5′-ACTG…CAGT-3′) in viral DNA and removes the 3′-CA dinucleotide (called 3′-processing step), before transferring the viral DNA into host DNA by creating a specific incision to host chromosomal DNA (called strand-transfer step). The monophosphate **1.79** inhibited this strand transfer step (IC_{50}: 68 µM) and **1.81** inhibited both the 3′-processing and strand transfer steps (IC_{50}: 75 µM and 53 µM, respectively).[62]



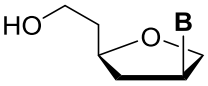
1.82



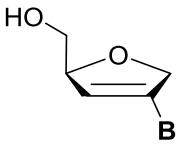
1.83



1.84



1.85: B = A, C, G

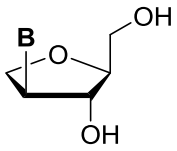


1.86: B = A, T

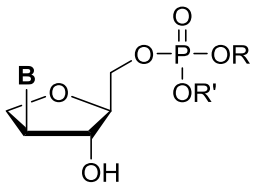
BVisoddU **1.82** inhibited HSV-1 replication at concentrations between 6 and 30.3 µM, which is almost 100 times higher than BVDU. Like BVDU, **1.82** is not active against HSV-2 strains, VV, VZV, VSV and CMV. Since BVDU and BVisoddU express relatively similar binding strengths to thymidine kinase, it is inferred that the difference in activity is probably related to different effects on HSV-1 DNA polymerase. [63] Nair and co-workers described the synthesis of enantiomeric compounds **1.83** and **1.84**,[64] as well as a homologue of the potent anti-HIV compound isoddA **1.85** [65] and 2′,3′-vinylic **1.86** [66]. None of these significantly inhibited HIV.

Inhibition of HSV-1 by **1.87a** and **1.87b** was weak (IC_{50}: 124 and 68.5 µM, respectively), while **1.87b** also exhibited weak anti-HSV-2 activity (IC_{50}: 137 µM).

Interesting results were obtained for anti-proliferative effect on human promyelocytic leukemia (HL-60) cells (ED_{50}: 5.08, 1.6, 8.06 µM for **1.87a**, **1.87b** and **1.87c**, respectively). Also the corresponding monophophates displayed anti-leukemia activity: **1.88a** and **1.88c** were weak inhibitors (190, 170 µM) while **1.88b** and **1.88d** displayed ED_{50} values of 4.5 and 9.3 µM, respectively. Based on the ^{1}H-^{1}H coupling constant it was inferred that these compounds adopt a S-type conformation (in DMSO).[67] The triphosphates of **1.87** were readily accepted by many polymerases (thermostable) and acted as chain terminators.[68]

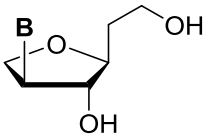


1.87a: B = A, **1.87b:** B = U,
1.87c: B = C,
B = T, 5I-U, hypoxanthine
8-chloroadenine

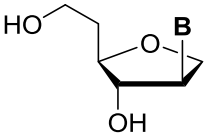


1.88a: B = *N*-benzoyladenine, R = *n*-butyl, R' = *o*-chlorophenyl
1.88b: B = *N*-benzoyladenine, R = *n*-octyl, R' = *o*-chlorophenyl
1.88c: B = uracil, R = *n*-octyl, R' = *o*-chlorophenyl
1.88d: B = uracil, R = *n*-octyl, R' = H

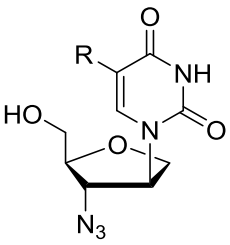
The isonucleoside 5′-homologue **1.89**, and its enantiomer **1.90** [69] and the AZT mimic **1.91** [70] failed to show promising anti-HIV activity.



1.89: B = A, G, T, U, C



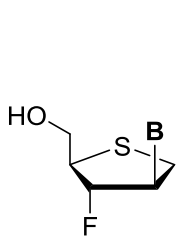
1.90: B = A, G, T, U, C



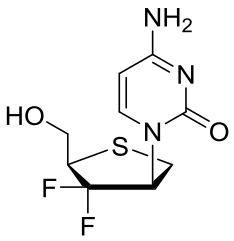
1.91: R = H, CH_3

The 3′-F-4′-thio, isonucleosides **1.92** did not exhibit anti-HIV/HSV/EMCV activity. The cytidine anlaogue exhibited anti-VSV actitivity (EC_{50}: 38.5 µM) but this was combined with high cytotoxicity (CC_{50}: 59.3 µM) in HeLa cell. The activity profile of this compound is interesting since it inhibited the RNA virus VSV, but unlike lamivudine from which it was structurally derived, failed to inhibit HIV.[71] For compound **1.93** an isostere of 3TC no biological activity could be found.[72] The locked nucleosides **1.94**, designed as S-Type conformers of lamivudine, did not show anti-

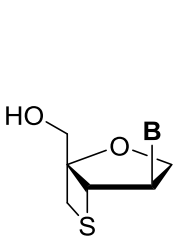
HIV-1 or HSV-1 activity.[73] The synthesis of diastereomers **1.95** is reported without biological evaluation.[74]



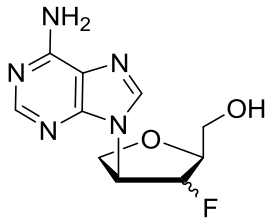
1.92: B = U, T, C



1.93

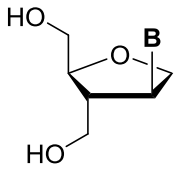


1.94: B = T, A

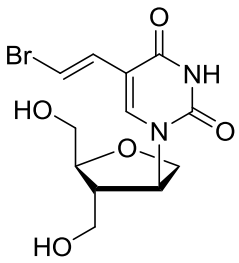


1.95

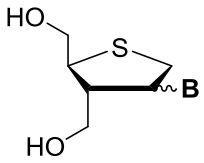
Interesting results were obtained with the ring expanded oxetanocin analogues **1.96**.[75,48] The adenine congener **1.96a** displayed potent broad spectrum antiviral activity, comparable with ganciclovir, HCMV (EC_{50}: 4.4 µM), HSV-1/2 (EC_{50}: 33.9, 37.7 µM), HBV (EC_{50}: 2.6 µM), and against cancer cell lines (L1210, IC_{50}: 10.2 µM; KB cells, IC_{50}: 196 µM). The guanine analogue **1.96c** showed selective inhibition towards HSV-1/2 (EC_{50}: 7.8-10 µM). Compounds **1.96b** and **1.96d** displayed EC_{50} values against HSV1/2 and HCMV in the 50-120 µM range, while **1.96b** also showed anti-HBV activity (EC_{50}: 23.5 µM).



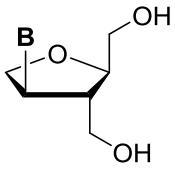
1.96a: B = A
1.96b: B = 2,6-diaminopurine
1.96c: B = G (BMS 181164)
1.96d: B = C



1.97: BMS 181165



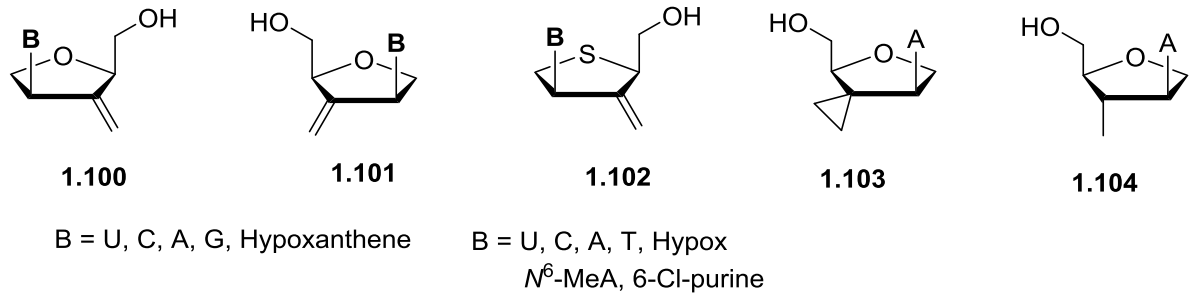
1.98: B = A, G,



1.99: B = T, A

The bromovinyluridine analogue **1.97** displayed very potent and selective inhibition of different strains of HSV and VZV (submicromolar to double digit nanomolar concentrations) without cytostatic effect, thereby being more effective than acyclovir against VZV. Because of its activity against simian varicella virus, extensive animal studies were carried out in African green monkeys, but further development was apparently stopped.[76] The 4′-thio counterpart of **1.96** was evaluated as an epimeric

mixture (**1.98**) for anti-HIV activity and found inactive.[77] Only the L-thymine analogue **1.99** exhibited moderate anti-HIV activity (IC_{50}: > 200 µM, EC_{50}: 0.8 µM, TI_{50}: > 250).[78]

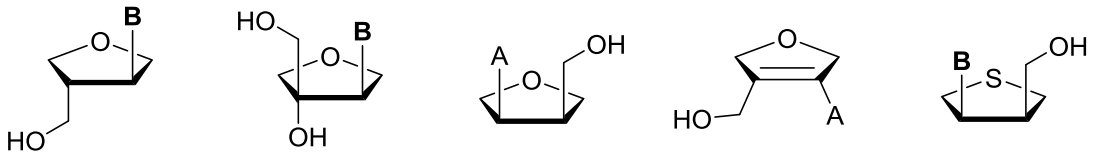


1.100 **1.101** **1.102** **1.103** **1.104**

B = U, C, A, G, Hypoxanthene

B = U, C, A, T, Hypox
N^6-MeA, 6-Cl-purine

In general exocyclic methylene compounds (**1.100-1.102**) and spiro-cyclopropyl **1.103** lack anti-HIV activity. Since these molecules are more rigid, diminished anti-HIV activity is not surprising. The compound series **1.100** are inactive against HIV-1 and HSV-1/2, but showed good activity against HCMV and surprisingly, also against HBV. The uracil derivative of **1.100** was more than 10-fold less activite against HCMV (EC_{50}: 47.3 µM) than ganciclovir in AD-169 cells. The adenine derivative of **1.100** expressed moderate activity against HCMV (EC_{50}: 134.7 µM) in addition to anti-HBV activity (EC_{50}: 1.5 µM). Their enantiomer series **1.101** did not display any activity.[79] The thio-sugar with 6-chloropurine base **1.102** exhibited very weak anti-HCV activity. All other nucleosides of the series were inactive against HIV-1, HBV, HCV, and HCMV.[80] Likewise, the spironucleoside **1.103** and 3′-α-methyl isonucleoside **1.104** lacked activity against HIV-1, HSV, VZV and CMV.[81]



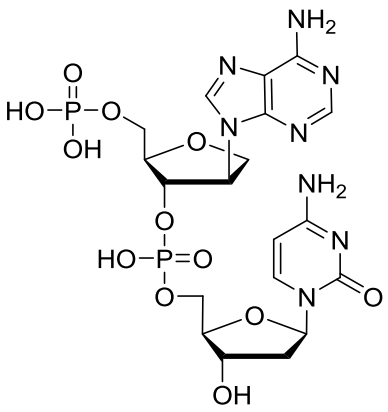
1.105 **1.106** **1.107** **1.108** **1.109**

B = A, C
5-F-C

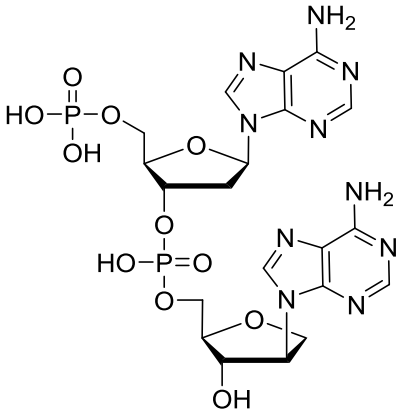
B = A, C
6-Cl-purine

B = T, C, U
5-F-U

Antiviral evaluation of a series of 3′-carbon substituted isonucleosides **1.105-1.108** indicated that they were marginally active or inactive against HIV-1, HSV, VZV and CMV.[82] For the thio-compound **1.109** no biological data have been reported.[83]



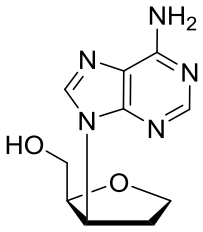
1.110



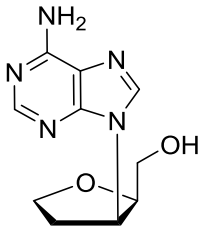
1.111

The dinucleotide **1.110**, composed of a natural nucleoside and an isonucleoside (pIsodApC), is reported to inhibit HIV integrase. It curtailed both 3′-processing and strand transfer steps (IC_{50}: 19 and 25 µM, respectively) in comparison to known natural dinucleotide pdApdC (IC_{50}: 6 and 3 µM, respectively). Interestingly, pdApIsodA **1.111** reduced the efficiency of the integrase enzyme only in the strand transfer step (IC_{50}: 41 µM).[84]

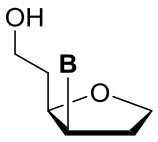
The synthesis of 3′-isonucleoside of adenine **1.112** and its enantiomer **1.113** is reported, but no biological activity data.[85] The anti-HIV activity of homologated nucleosides of the structure **1.114** is not significant,[86] while no biological data have been released for **1.115**.[87]



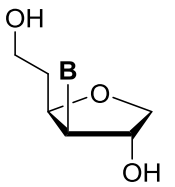
1.112



1.113

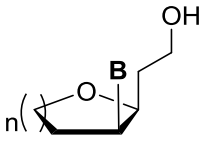


1.114: B = A, T U, C



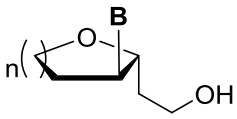
1.115: B = T, U 5F-U

Also, antiviral data of the base transposed furanose and pyranose nucleosides **1.116** and **1.117** are awaited,[88] while compound **1.118** did not display anti-HCV activity.[89]



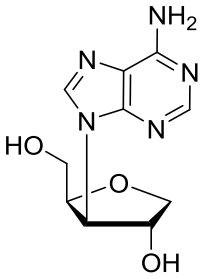
B = A, n=1
B = T, n=1
B = T, n=2

1.116



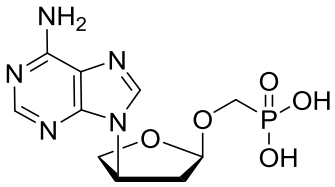
B = A, n=1
B = T, n=1
B = T, n=2

1.117

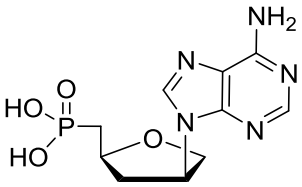


1.118

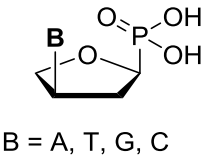
1.3.2.4. Isonucleoside phosphonates



1.119



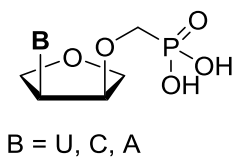
1.120



B = A, T, G, C

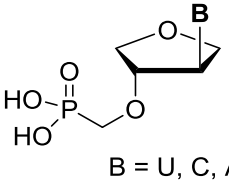
1.121

The isodideoxyadenosinephosphonate (isoddAP, **1.119**) demonstrated relatively potent anti-HIV activity, though its EC_{50} (9.5 µM) is almost two and half times higher than that of tenofovir, which is attributed to poorer activation to the triphosphate. This phosphonate possessed a rather attractive profile towards mutant strains. The activity dropped by a factor eleven in M184V RT, and a factor of 3.2 and 2.2 against 6TAMs (thymidine analogue mutation) and K65R mutant strains, respectively. This is in sharp contrast to tenofovir but in line with abacavir. The extrusion of pyrimidine based nucleosides by thymidine analogue mutation is well documented with adenine based acyclic nucleosides being most vulnerable to these mutations.[90] Phosphonate **1.120** showed much lower activity compared to the parent compound (*S*,*S*)-isoddA.[91] Derivatives **1.121** did not show *in vitro* antiviral activity.[92]



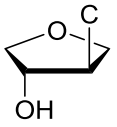
B = U, C, A

1.122

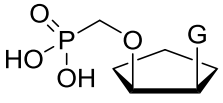


B = U, C, A

1.123



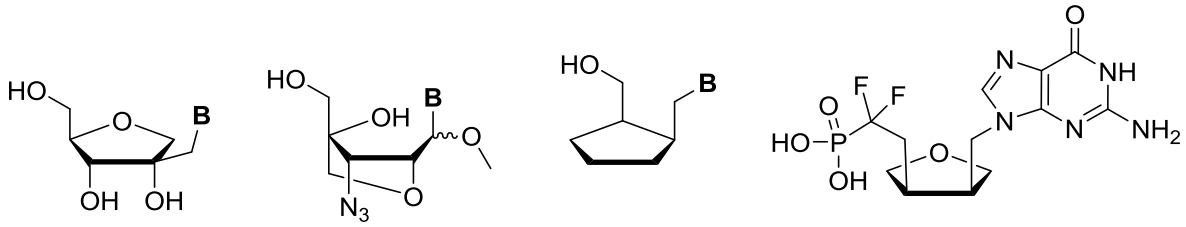
1.124



1.125

Compounds **1.122**–**24** were only screened against HCMV, but only **1.124** proved active (IC_{50}: 15.2 µM; CC_{50}: 507.1 µM).[93] The carbocyclic guanine phosphonate **1.125** showed reasonable activity against HIV in CEM cells (IC_{50}: 20 µM).[94]

1.3.2.5. Homonucleosides and homonucleoside phosphonates



1.126
B = U, C, A, G

1.127
B = T,C,A

1.128
B = 6-Chloropurine

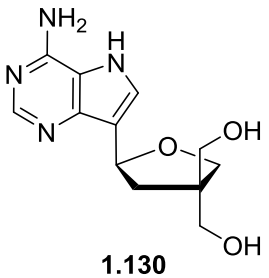
1.129

Idenix Pharma reported the synthesis of 2′-C branched nucleosides **1.126**,[95] but these were inactive against HCV, HIV and other viruses and not cytotoxic. The lack of activity was explained as, the entropy price paid by the addition of rotational flexibility between the sugar and the nucleobase or the change in spatial distance between the base and the primary alcohol of these isohomonucleosides. The locked AZT mimic **1.127** lacked anti-HIV-1 activity as well as toxicity in MT-4 cells.[96] The 1,2-disubstituted carbocyles **1.128** [97] showed antitumor activity (IC_{50}: 52.1, 24.3, 59.5 mM against cell lines L1210/0, Molt4/C8, CEM/0, respectively) and its pyrimidine analogues exhibited marginal anti-HIV-1/2 activity (<285 µM). Compound **1.129** is reported to be potent inhibitor of human purine nucleoside phosphorylase (PNP), but no antiviral activity data have been reported.[98]

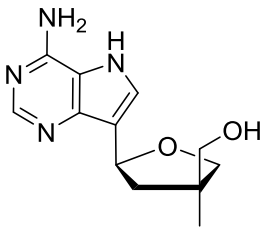
1.3.2.6. C-nucleosides and extremely modified non-natural bases

The L-apio C-nucleosides **1.130** and **1.131** revealed moderate antiviral profiles.[99] Compound **1.131** (EC_{50}: 20.5 µM) performed somewhat better than **1.130** (EC_{50}: 35.2 µM) against HIV-1. Both exhibited moderate to poor activity against HSV-1/2 and HCMV (EC_{50}: 94.6 - 190 µM). Compound **1.132** and **1.133** showed moderate activity

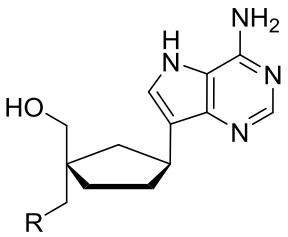
against HIV-1 (EC_{50}: 25.1 and 14.7 µM, respectively) in MT-4 cells without any cytotoxicity up to 100 µM.[100]



1.130

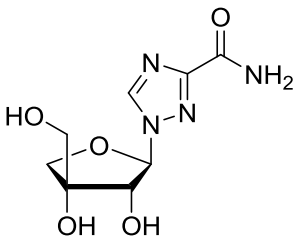


1.131

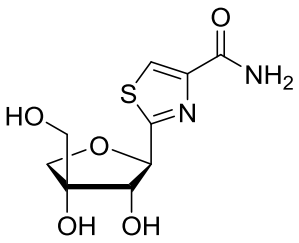


1.132: R = OH
1.133: R = H

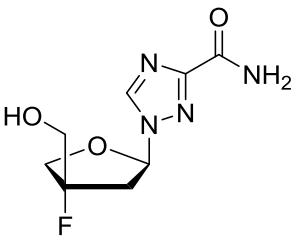
Kim *et al.* synthesized the ribavirin type apionucleosides **1.134** and its thiazole analogue **1.135**. Both compounds were screened against a panel of viral strains without notable activity. Compound **1.136**, its enantiomer and diasteriomers are claimed to be a potent anti-HBV agents but no exact data are available.[101] The isonucleosides featuring modified bases such as triazolyl-4,5-dicarboxamide (**1.137)**,[102] and imidazolyl-4-carboxamide **1.138** were inactive or showed very low potency against HIV.[61]



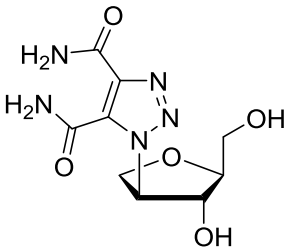
1.134



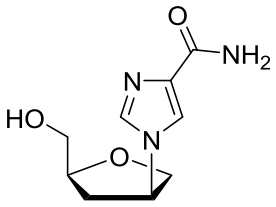
1.135



1.136



1.137



1.138

1.4. Nucleosides as A_3 Adenosine Receptor (AR) Ligands[103]

Transmembrane receptors coupled to G-proteins (heterotrimeric guanine nucleotide binding proteins) constitute a large receptor family, often referred to as G-protein coupled receptors (GPCRs) or seven-transmembrane domain (7TM) receptors. The amino terminal part of these receptors is located extracellularly and these receptors pass 7 times through the cell membrane, thereby forming seven transmembrane helices, three or four intracellular loops (ILs), three extracellular loops (ELs) and an intracellular C-terminus.[104]

Triggered by messenger molecules or signals outside the cell, GPCRs activate different signal transduction pathways inside the cell and, ultimately, cellular responses. The extracellular messengers range from photons over aminergic neurotransmitters to proteins. GPCRs are ubiquitous and involved in processes varying from directed chemotaxis[105] of small organisms (*e.g.* searching food for survival) to triggering apoptosis (cell death) in large animals. They are the targets of almost 50% of marketed active pharmaceutical ingredients.[106]

GPCRs activated by extracellular adenosine are classified as adenosine receptors (ARs), which are divided in four different subtypes, *i.e.* A_1, A_{2A}, A_{2B} and A_3 adenosine receptors.[107,108] The amino acid sequence similarity between the human (h) A_3AR and hA_1, hA_{2A}, hA_{2B}AR is 54 %, 48 % and 44 %, respectively.[103b] The ARs use different signaling pathways. The A_1AR and A_3AR are coupled to Gi-proteins, and upon activation lead to adenylate cyclase inhibition, while the A_{2A}AR and A_{2B}AR are coupled to Gs-proteins and lead to adenylate cyclase activation. In some cells (*e.g.*, mast cells), the A_{2B}AR is dually coupled to Gs and Gq and consequently also elevates phosphoinositides, mobilizes calcium and activates phospholipase C and MAPK.[107,109]

Besides adenosine **1.139** itself, which is used clinically for the treatment of supraventricular tachycardia, [110] only one adenosine receptor-specific agent – the A_{2A}AR agonist regedenoson (**1.140**) – has so far been approved by the FDA. However, a relatively large group of AR ligands are currently under clinical evaluation (table 1.1).[111]

Table 1.1. Adenosine receptor ligands in clinical use or trails (Figure 1.6).

Ligand, Subtype and action		***Application***	***Phase***	***Company***
*Adenosine **1.139** (Adenocard)*	*A_1 agonist*	*Paroxysmal supraventricular tachycardia*	*Approved*	*Astellas*
INO-8875		*Glaucoma*	*I–II*	*Inotek*
*Capadenoson **1.140**, Bay-68-4986*		*Atrial fibrillation*	*II*	*Bayer-Schering*
*Adenosine **1.139** (Adenoscan)*	*A_{2A} agonist*	*Myocardial perfusion imaging*	*Approved*	*Astellas*
*Apadenoson **1.141**, ATL146e*		*Myocardial perfusion imaging*	*III*	*Forest Laboratories*
(Stedivaze)				
*Regadenoson **1.142**, CV-3146 (Lexiscan)*		*Myocardial perfusion imaging*	*Approved*	*Astellas/Gilead*
*Regadenoson **1.142**, CV-3146 (Lexiscan)*		*Sickle cell disease*	*I*	*Dana-Farber Cancer Institute*
*IB-MECA **1.143**, CF101*	*A_3 agonist*	*Rheumatoid arthritis, psoriasis, dry eye, glaucoma*	*II/III*	*Can-Fite*
*Cl-IB-MECA **1.144**, CF102*		*Hepatocellular carcinoma, chronic hepatitis C (genotype 1)*	*II*	*Can-Fite*
*Caffeine **1.145***	*AR antagonist*	*Sleep apnea, cancer pain, PD*	*II/III*	*Univ. of Texas, McMaster Univ., Nobelpharma, Korea Research, McGill University*
*Theophylline **1.146***		*Asthma, COPD*	*Approved*	–
*Istradefylline **1.147**, KW-6002*	*A_{2A} antagonist*	*PD*	*III*	*Kyowa Hakko*
KW-6356		*PD*		*Kyowa Hakko (in Asia), Lundbeck*
*Preladenant **1.148**, SCH-420814*		*PD*	*III*	*Schering*
*Tozadenant **1.149**, SYN-115*		*PD, cocaine dependence*	*IIB*	*Biotie, NIDA (Synosia Therapeutics)*
*ST-1535 **1.150***		*PD*	*I*	*Sigma-Tau*
V81444		*PD*	*I*	*Vernalis*
*[^{11}C]-SCH442416 **1.151***		*PET imaging of PD*	*I*	*Institute for Neurodegenerative*
*[^{123}I]MNI-420 **1.152***		*SPECT imaging of PD, Huntington's disease*	*I*	*Disorders*

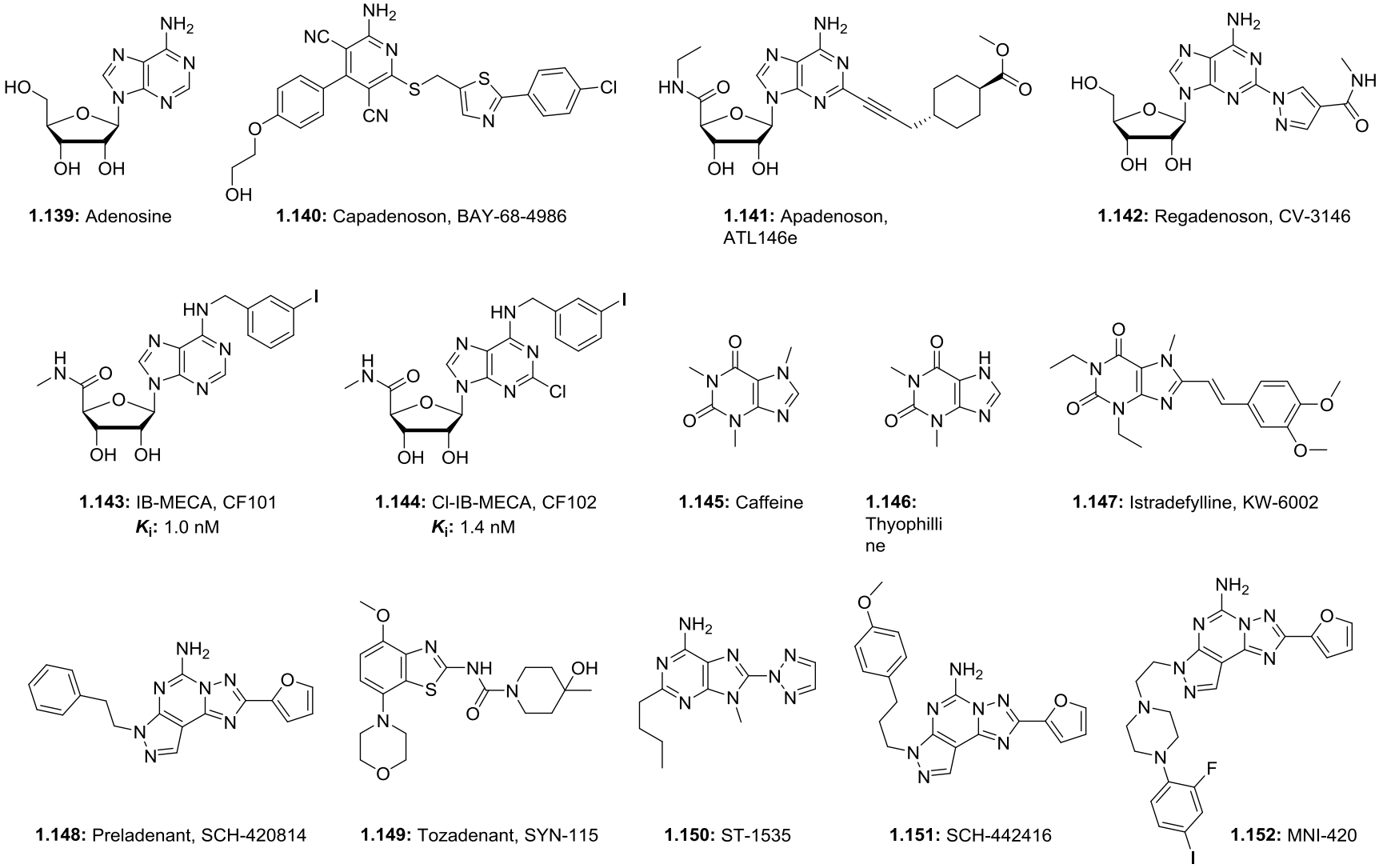


Figure 1.6. Structure of adenosine receptor ligands in clinical use or in trails.

The A_3AR agonists are found effective against inflammatory conditions such as rheumatoid arthritis, psoriasis, keratoconjuctivitis sicca (dry eye syndrome), and glaucoma, while antagonists are potentially useful for the treatment of asthma. The detection of A_3AR expression in cancer cells suggests a potential use of turning selective ligands in to diagnostic tools, while a number of recent studies have shown that activation of this receptor attenuates proliferation of melanoma, colon, and prostate cancer cells and may also inhibit cancer metastasis. The observation that the upregulation of *ADORA3* mRNA expression in PBM cells from patients with hepatocellular carcinoma compared to healthy individuals could lead to its use as potential biomarker for the detection of disease progression.[112]

Surprisingly, knockout of the A_3 receptor in mice resulted in marked phenotypes even at locations where the receptors are very sparse (*e.g.*, brain). A possible explanation for this is that A_3ARs may have a role in development.[113]

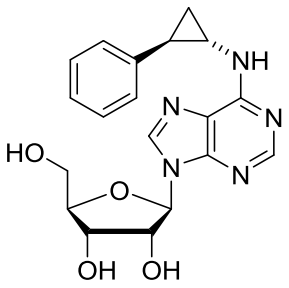
1.4.1. Substitution patterns on adenosine and their effect on A_3AR modulation

Previous medicinal chemistry studies demonstrated that an adenosine derivative's ability to activate the A_3AR is more structure sensitive than at other AR subtypes.[114] While a number of changes in various regions of the adenosine molecule have been shown to enhance binding to the A_3AR, these usually tend to reduce A_3AR efficacy, leading to nucleoside analogues that are partial agonists or antagonists.

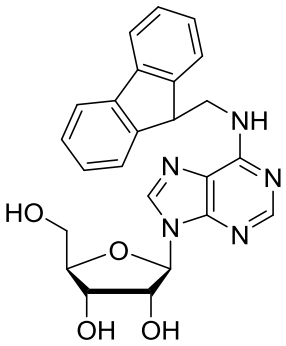
Structure-affinity and structure-efficacy relationships at this subtype, showed that N^6 substitution with selected benzyl groups, introduction of a 2-chloro substituent and conformational constraints of the ribose moiety of adenosine all contribute to affinity enhancement but at the expense of efficacy. In most cases the lost efficacy may be regained by substituting the 4′-hydroxymethyl group to a 4′-*N*-methyl or ethyl carboxamide.

1.4.1.1. Modification to adenine base

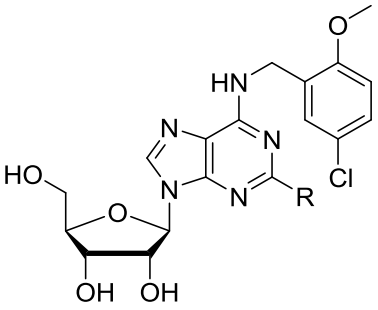
The N^6 and C2 modifications in combination with sugar variations are the most explored in search of potent and selective AR ligands. The presence of a hydrogen on N^6 is generally essential for high affinity agonists, but a C6-CF_3 antagonist is known.[115] Likewise, C8-substitution (as in 8-alkynylated derivatives) generally leads to antagonists.[116] Many A_3AR ligands feature a large substituent at N^6 and a small group on C2 (H, Cl, CN, N_3, etc.), or *vice versa*, a small substituent at N^6 (CH_3, OCH_3, Et, etc.) and a large group at C2. A notable exception is N^6-methyl-2-cyanoadenosine with two relatively small substituents at N^6 and C2, a potent agonist for the human A_3AR (K_i: 3.4 nM) that is inactive at rat A_3AR.[117] Well-known N^6-phenethyl substituents are found in compounds **1.153** and **1.154**,[118] which are potent but non-selective (A_1AR) A_3AR agonists. Small substituents at C2 (Cl, CN, N_3) generally increase A_3AR selectivity at the expanse of affinity (and/ or efficacy) as exemplified by the couples **1.143** and **1.144** and analogues **1.155-1.157** from our laboratory.[117,119]



1.153: K_i: 0.63 nM

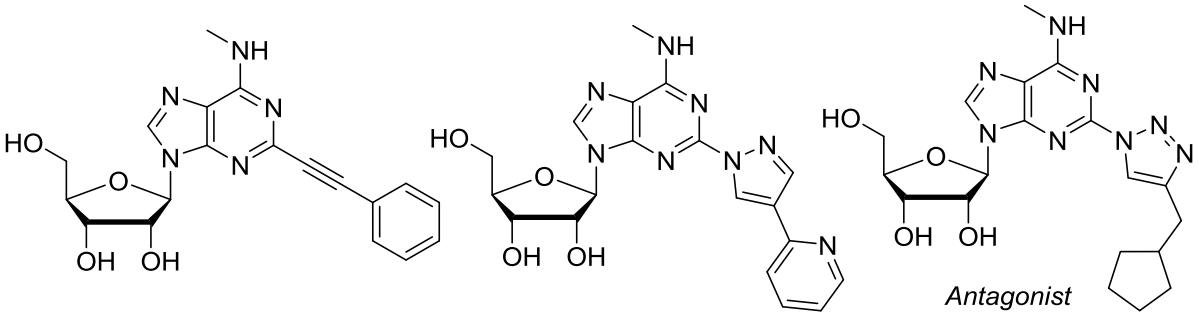


1.154: K_i: 0.91 nM



1.155: R = H, K_i: 1.3 nM
1.156: R = N_3, K_i: 1.4 nM
1.157: R = CN, K_i: 2.8 nM

Various 2-ethynyl modifications produce potent A_3AR ligands (*e.g.*, compound **1.158**),[120] whose A_3 selectivity may be increased by alterations at 5′-position.[121] C2-heterocycle substituted analogues form an interesting class of potent A_3AR ligands. The C2-pyrazol-4-yl analogue **1.159** is the most selective agonist of this class,[122] 4-substituted triazol-1-yl analogue **1.160**, synthesized in our lab, turned out to be a potent antagonist.[119]

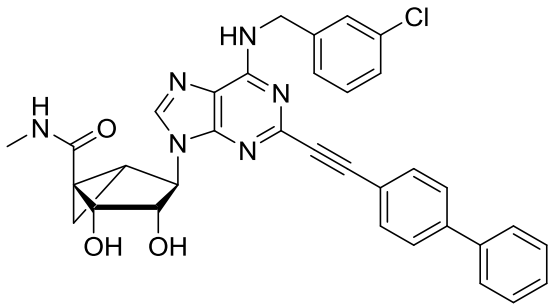


1.158: K_i: 3.4 nM

1.159: K_i: 2.0 nM

Antagonist
1.160: K_i: 1.3 nM

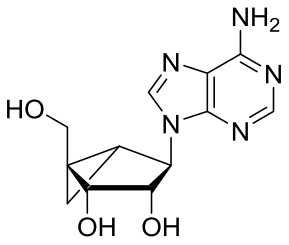
While previously regarded as incompatible, in recent years a number of highly potent and selective A_3AR modulators have been described that combine two bulky moieties at N^6 and C2. By structure-based design, Tosh *et al.*, for example, discovered compound **1.161** as a potent and selective A_3AR agonist. The C2 substituent is predicted to penetrate deeply into the TM2 region and to act as a firm anchor.[123]



1.161: K_i: 3.1 nM

1.4.1.2. Sugar and sugar attachments

Most interactions with the sugar are believed to be indispensable for high affinity. For hA_3AR agonistic activity, a 4′-*N*-methylcarboxamide is the most popular sugar modification, known to increase affinity and efficacy. Replacing the 4′-oxygen by sulfur increases the affinity and selectivity. It has become increasingly evident that A_3ARs prefer slightly rigid substrates with directed interactions. For example, the northern conformation locked (N)-methanocarba adenine **1.162** (K_i: 404 nM) is 150 times more potent than its southern conformer **1.163**.[124] It is observed that these N-locked compounds tend to show low efficacy but altering 5′- hydroxyl to uronamide allows regaining efficacy as demonstrated for **1.164**[125] compared to **1.143** or **1.144**.[126]



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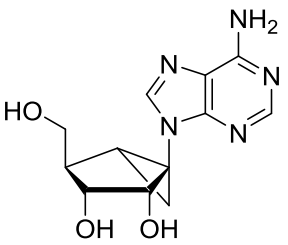

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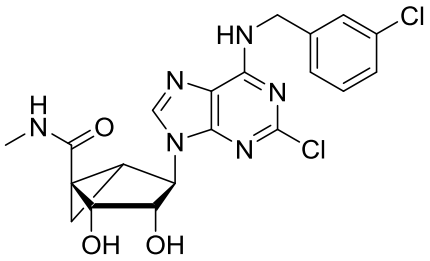
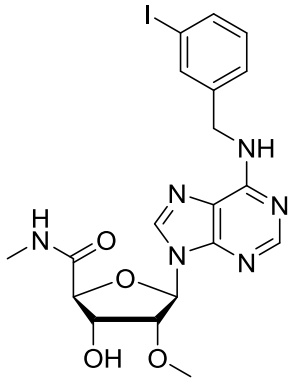
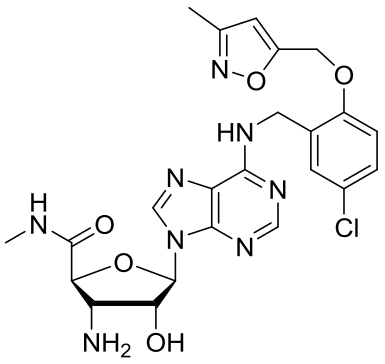


1.164: K_i: 0.29 nM

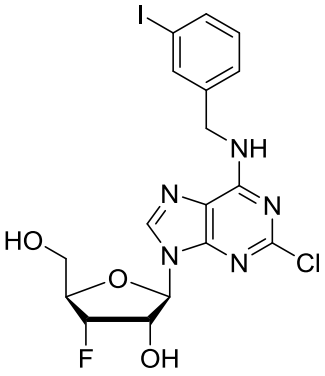
The similar behavior of compounds **1.165** and IB-MECA (**1.143**) as A_3AR agonists suggests that the 2′-OH group in **1.143** probably acts as a hydrogen bond acceptor and that only one hydroxyl may suffice provided the sugar is in correct conformation.[127] The 3′-amino compound **1.166**, a potent agonist with an ideal pharmacological profile, high selectivity, and acceptable aqueous solubility, suggests that the 3′-substituent acts as hydrogen bond donor.[128] The 2′-deoxy-2′-fluoro compound **1.167** is an antagonist, with moderate affinity.[129]



1.165: EC_{50}: 1.9 nM

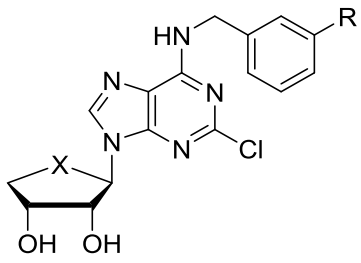


1.166: K_i: 0.58 nM



Antagonist
1.167: K_i: 75 nM

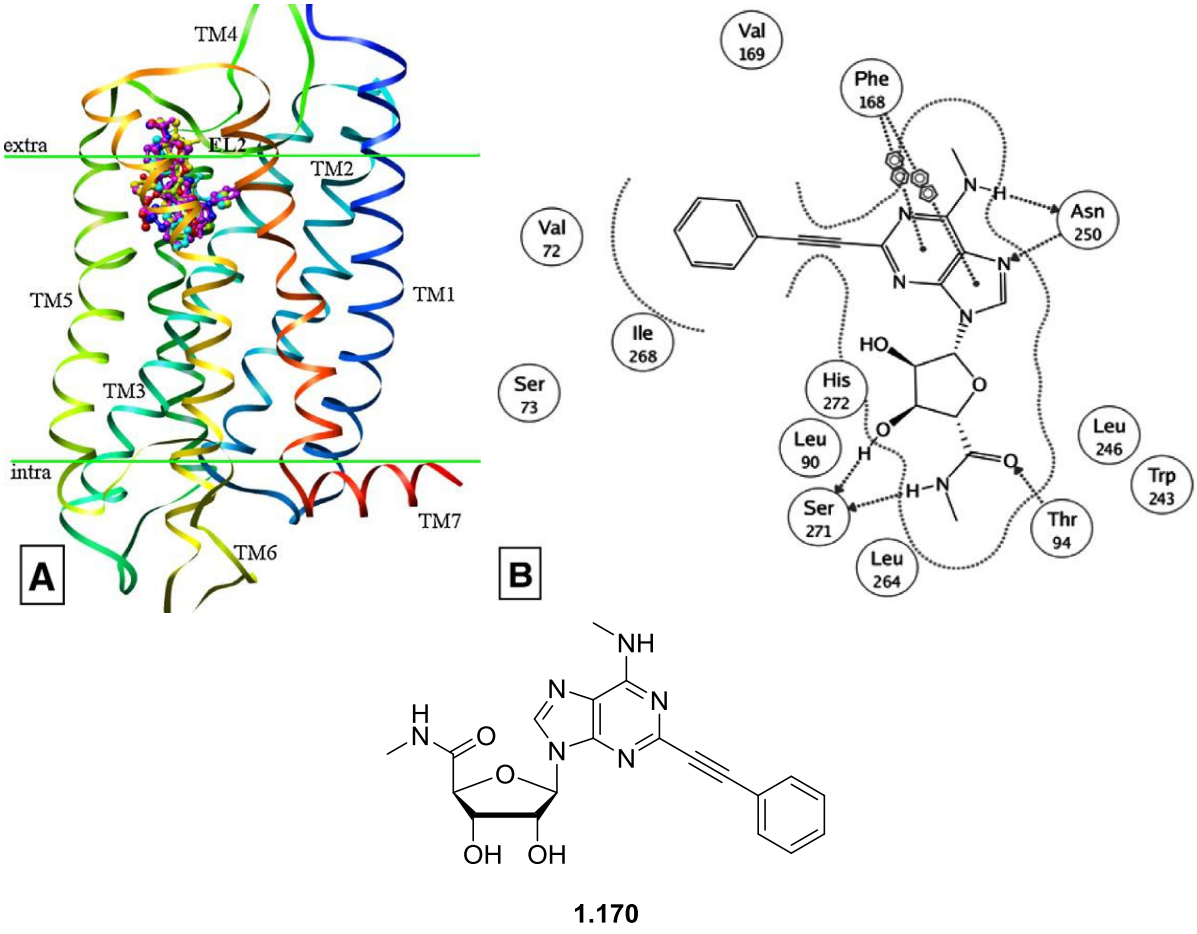
In the context of the envisaged analogues it is noteworthy that the 5′-CH_2OH-deleted compounds **1.168** and **1.169**[130,131] exhibit potent and A_3AR selective antagonistic properties.



Antagonists
1.168: X = O; R = Br; K_i: 13 nM
1.169: X = S; R = Cl; K_i: 1.7 nM

1.4.2. A_3AR homology modelling

The development of ligands for the A_3AR has been directed mainly by traditional medicinal chemistry, but the influence of structure-based approaches is increasing. Rhodopsin-based homology modelling had been used for many years to obtain three-dimensional models of the A_3AR, and to predict interactions with A_3AR ligands with different chemical scaffolds. The recently published structure of the human $A_{2A}AR$ and other GPCRs provide new templates for creating A_3AR homology models, which may be helpful in providing structural hypotheses for the design of new ligands.[132] Site-directed mutagenesis of the A_3AR shows an important role in ligand recognition for specific residues in TM3, TM6 and TM7.



1.170

Figure 1.7. (A) Ligand binding site[133] (B) Predicted interactions of agonist **1.170** with A_3AR.[134]

Using homology modelling, the binding mode of compound **1.170** in the A_3AR was predicted.[134] The ligand recognition site is in the upper part of the receptor (Figure 1.7 [A]), between trans-membrane domains (TMs) and the extracellular loops (ELs). TMs 3, 5, 6, 7 and EL2 are believed to play an important role in ligand recognition. TM4 is in direct contact with TM3 but not involved in ligand binding, while TMs 1 and 2 are far away from the binding site. In recent studies, however, TM2 was explored in drug-design efforts, *e.g.* in the design of compound **1.161**. Some of the essential interactions of receptor with ligands are listed below (Figure 1.7 [B]):

- Conserved Asn250 (6.55) makes two important hydrogen bonding interactions, one as H-bond acceptor with N^6 – H and the other H-bond donor to N^7.
- Stacking interaction between conserved Phe168 (EL2) and adenine ring.
- Thr94 (3.36) interaction via H-bonding to 5′-OH/NH (interaction responsible for efficacy).
- Ser271 (7.42) and His272 (7.43) make crucial H-bonding interactions with the 3′ and 2′ substituents (hydroxyls) respectively.
- Interactions with hydrophobic pockets in the regions involving highly conserved Leu90 (3.32), Leu246 (6.51) and Ile268 (7.39).

1.5. Nucleosides as Inhibitors of *Mycobacterium Tuberculosis* Thymidylate Kinase[135]

Nearly two million people die from tuberculosis (TB) every year, and this disease is second only to AIDS as the greatest killer worldwide to a single infectious agent. In humans is caused by *Mycobacterium tuberculosis*. The prevalence of TB with HIV, multidrug and extreme drug resistance is a major and growing global threat. The situation asks for new drugs preferably acting on unaddressed targets[136] or new concepts (*e.g.* quorum sensing) with short duration of treatment. Thymidine monophosphate kinase (TMPK/ ATP-dTMP phosphotransferase) was recently introduced as an interesting target enzyme.[137,138] The TMPK catalyzes the conversion of deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate using ATP as a phosphate source. TMPK is situated at the junction of *de novo* and salvage pathways of thymidine triphosphate (dTTP) biosynthesis and is the last specific enzyme in these pathways.

1.5.1. Structure of TMPK*mt*

The primary sequence similarity between human TMPK (TMPK*h*) and TMPK*mt* is 22%. Despite the fact that the global folding of all TMPKs are similar, their active site and their substrate preferences are unique. The monophosphate of AZT, for example, is an inhibitor of TMPK*mt* (K_i: 10 μM), while acts a substrate of its mammalian counterpart.[139] This observation opens opportunities for designing specific inhibitors using AZT-MP as a template. The important regions for the catalytic activity of the enzyme are revealed by the structure of its complex with the natural substrate dTMP.[140]

The P-loop motif [141] (residues 7-14, $GlyX_1X_2X_3X_4GlyLysX_5$) is an important segment which positions the phosphoryl groups of the phosphate donor through hydrogen bonding interations between the α and β ATP phosphate oxygens and amide backbone hydrogens. Asp9 (at position X_2 of the P-loop motif), a highly conserved amino acid,

acts as firm anchor between the P-loop and the phosphoryl acceptor dTMP via a hydrogen bonding between its carboxylic acid residue and the 3′-hydroxyl group of dTMP. Any mutation to D9 residue (or equivalent in other species, *e.g.* D15 in TMPK*h*) across all sections of TMPK abolishes the phosphoryl transfer ability.[142]

The DR(Y/H/F) motif contains the strictly conserved arginine residue and serves as a clip that properly aligns the 'to be transferred' γ-phosphate of ATP and the acceptor phosphate of 5′- dTMP, in such a way that is favorable for interaction with arginine of LID region to effect phosphate transfer.

The LID region (residues 147-159) is like a tethered cap in all nucleotide monophosphate kinases. The binding of ATP exerts substantial conformational change[143] and brings the catalytic arginines (of the LID region) to the reaction center.

Some subtle but important differences between TMPK*mt* and TMPK*h* make the former an interesting target.

- The LID region in TMPK*h* does not contain positively charged amino acids like in TMPK*mt*, which instead reside in the P-loop motif with a single mutation to lysine19 and regular conserved argenines.
- TMPK*mt* unlike its counterparts has positively charged residues in both LID and P-loop (Arg14).
- The LID closure of TMPK*mt* is largely due to the binding of a Mg^{2+} ion in the dTMP-binding site and not to the binding of ATP.[144]
- The difference in affinity of dTMP (K_m: 4.5 μM) and ATP (K_m: 100 μM) for TMPK*mt* is more than 20 fold, suggesting that initial dTMP binding is largely favored.[139]
- In TMPK*mt* the magnesium ion lies in the dTMP-binding site, while in its human counterpart Mg^{2+} is typically found far away coordinating with phosphates of NTP.

Important interactions between dTMP and the TMPK*mt* enzyme are[139,145] (Figure 1.8):

- A stacking interaction between the pyrimidine nucleobase and Phe70.

- A hydrogen bond between O-4 of thymine and the Arg74 side-chain, which explains preference to thymidine over cytidine.
- A hydrogen bond between Asn100 and N-3 of the thymine ring.
- A network of hydrogen bonds between 3′- hydroxyl of dTMP (responsible for positioning the carboxyl of Asp9 to magnesium ion via water9 and the phosphate oxygen of dTMP).
- Hydrogen bonds and ionic interaction between the 5′-*O*-phosphoryl and Tyr39, Phe36, Arg95 and Mg^{2+} respectively.
- The presence of Tyr103 close to the 2′-position, which confers selectivity towards dTMP over its ribose analogue.

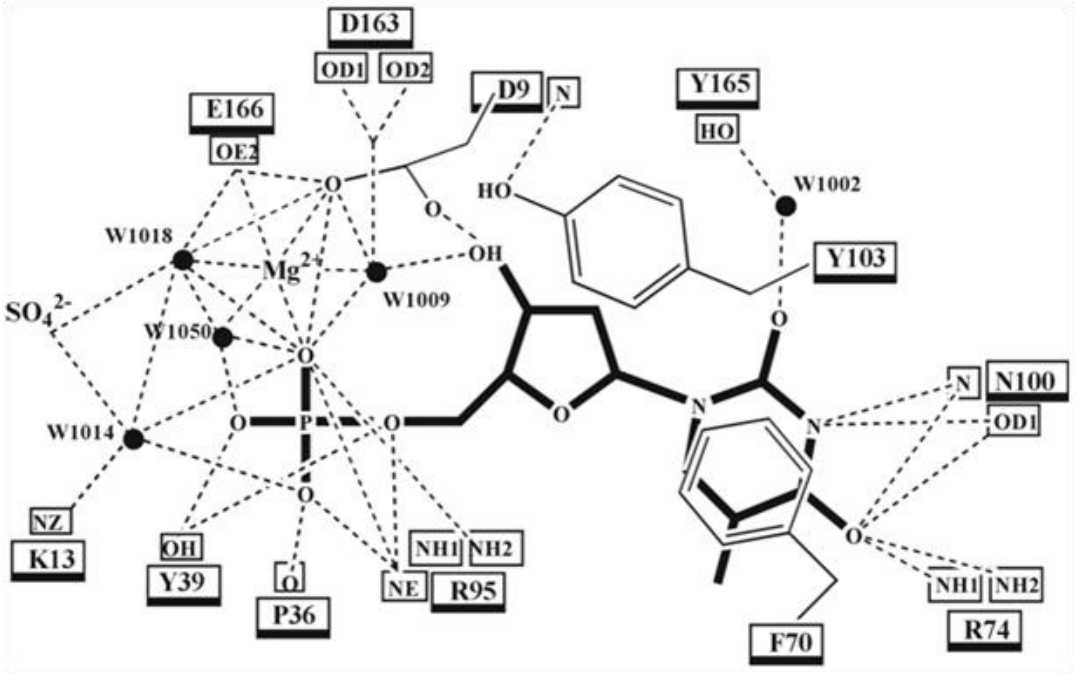
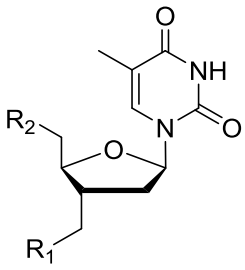


Figure 1.8. Schematic drawing of the dTMP (dark lines) binding site of TMPK*mt*.[139]

TMPK*mt* has broader substrate specificity for nucleoside triphosphates than other NMPKs. The reaction rates with ATP or dATP as phosphate donors are similar. Other efficient phosphate donors in order of efficacy are: ITP > GTP > CTP > UTP.[146]

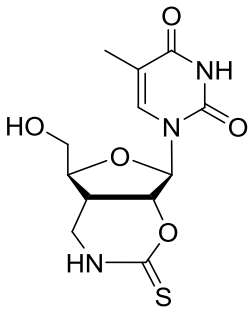
1.5.2. Sugar modified compounds

Based on AZT-MP as template Vanheusden *et al.* synthesized a series of 3′-C branched chain derivatives.[147] Among the synthesized compounds 3′-CH_2NH_2 (**1.173**), 3′-CH_2N_3 (**1.172**) and 3′-CH_2F (**1.174**) nucleotides exhibited the highest affinities with K_i values of 10.5, 12, and 15 μM, respectively. A major limitation of these monophsophates is that their charge is expected to impede diffusion through cell membranes. Hence, it was interesting to see that the corresponding nucleosides **1.175-8** exhibited TMPK*mt* affinities in the same order of magnitude and displayed superior selectivity vs. TMPK*h*.



1.171: R_1 = OH, R_2 = OPO_3^{2-}
1.172: R_1 = N_3, R_2 = OPO_3^{2-}
1.173: R_1 = NH_2, R_2 = OPO_3^{2-}
1.174: R_1 = F, R_2 = OPO_3^{2-}
1.175: R_1 = OH, R_2 = OH
1.176: R_1 = N_3, R_2 = OH
1.177: R_1 = NH_2, R_2 = OH
1.178: R_1 = F, R_2 = OH

Also compounds **1.179**, **1.180** and **1.181**, obtained by serendipity, exhibited interesting inhibition constants 3.5, 13.5 and 37 μM, respectively. Modelling studies revealed that the second (**II**) nucleoside residue of **1.181** occupies the position of ATP binding site in the enzyme (Figure 1.9).[148]



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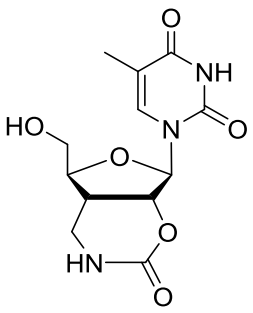
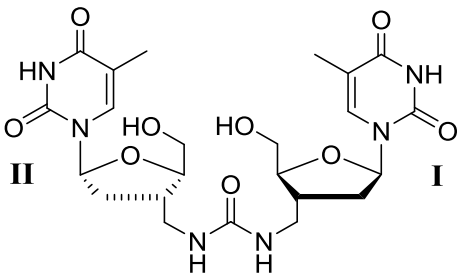


1.180



1.181

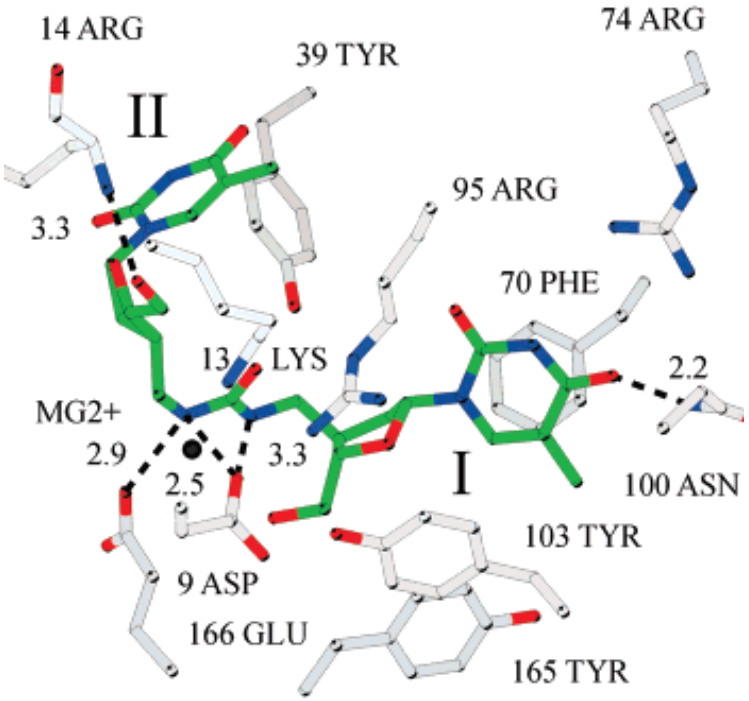
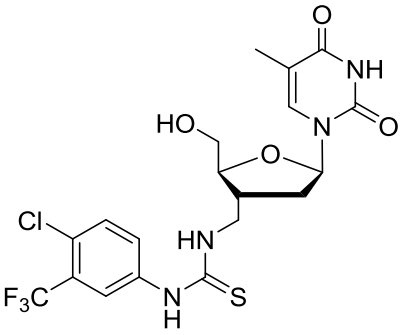
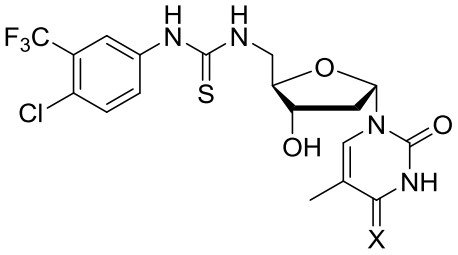


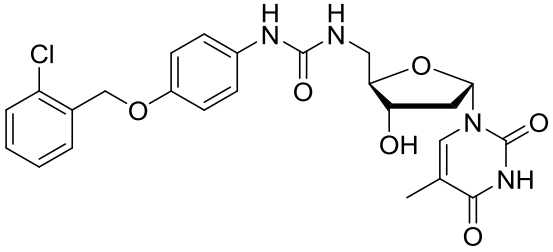
Figure 1.9. Predicted binding mode of **1.181**[148]



1.182: K_i: 5 μM



11.183a: X = O; K_i: 0.60 μM
11.183b: X = S; K_i: 0.17 μM

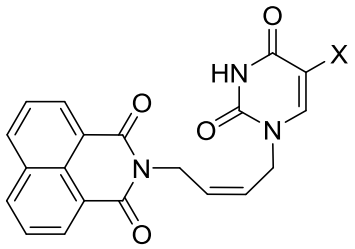


1.184: *Pf*TMPK - EC_{50}: 28 nM; CC_{50}: 29 μM

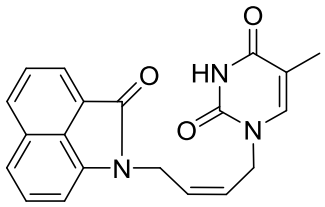
Based on the dimeric nucleoside **1.181**, compound **1.182** was synthesized and found to be a potent TMPK*mt* inhibitor.[149] Based on the predicted binding mode of **1.181** (Figure 1.9) 5′-modified α-thymidine analogue **1.183a**,**b** were synthesized, which are

the most potent nucleosidic TMPK*mt* inhibitors reported to date.[150] Using compound **1.183** as a starting point, Cui *et al.* recently designed, synthesized and evaluated a number of α-thymidines as *Plasmodium falciparum* TMPK inhibitors.[151] This work resulted in a compound **1.184** with potent antimalarial activity (EC_{50}: 28 nM; CC_{50}: 29 μM), although TMPK inhibition is probably not the only mode of action for this analogue.

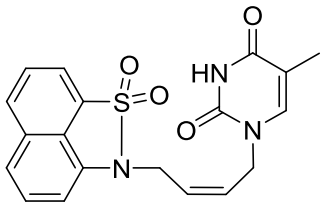
Familiar *et al.* reported the synthesis of acyclic compounds **1.185-8**, which were based on extensive modelling studies.[152] Out of 20 different spacers synthesized, a *Z*-2-butene as in **1.185**, **1.186** was chosen as an ideal linker. The naphtholactam analogue **1.187** and naphthosultam analogue **1.188** emerged as the most potent TMPK*mt* inhibitors. However, these analogues lacked antimycobacterial activity, probably due to poor solubility.



1.185: X = CH_3, K_i: 1.9 μM
1.186: X = Br, K_i: 1.1 μM



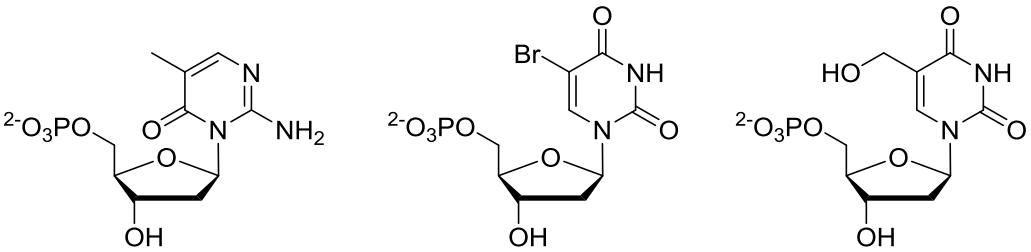
1.187: K_i: 0.42 μM



1.188: K_i: 0.27 μM

1.5.3. Base Modified compounds

5-MethylisoCMP analogue **1.189** is a weak inhibitor (K_i: 130 μM), dUMP is a poor substrate of TMPK*mt* with 50 fold reduced affinity (K_m: 2.1 mM), and a threefold lower rate than dTMP. All 5-halogen analogues were poor substrates, except 5-bromo dTMP (**1.190)** which had an affinity (K_m: 30 μM) and rate comparable to dTMP.[139] Hence, those substituents which can enhance the stacking interaction with Phe36 (*i.e.*, 5-bromovinyl, 5-furanyl, 5-benzyloxymethyl, 5-thienyl) or hydrogen bonding with Arg74 (5-CH_2OH-dUMP, **1.191**) were synthesized. Compound **1.191** was found to possess a K_i value of 110 μM while its α-anomer was fourfold less active.[153]



1.189 **1.190** **1.191**

The crystal structure of **1.191** (Figure 1.10)[153] shows hydrogen bonds between hydroxyl of 5-CH_2OH and water molecule (W12). The hydroxyl group may also take part in a network of interactions between E6, R95, Y179, and S99. These interactions in the dTMP binding site make 5-CH_2OH-dUMP a week inhibitor rather than a substrate, as was the case for 5-Br-dUMP.

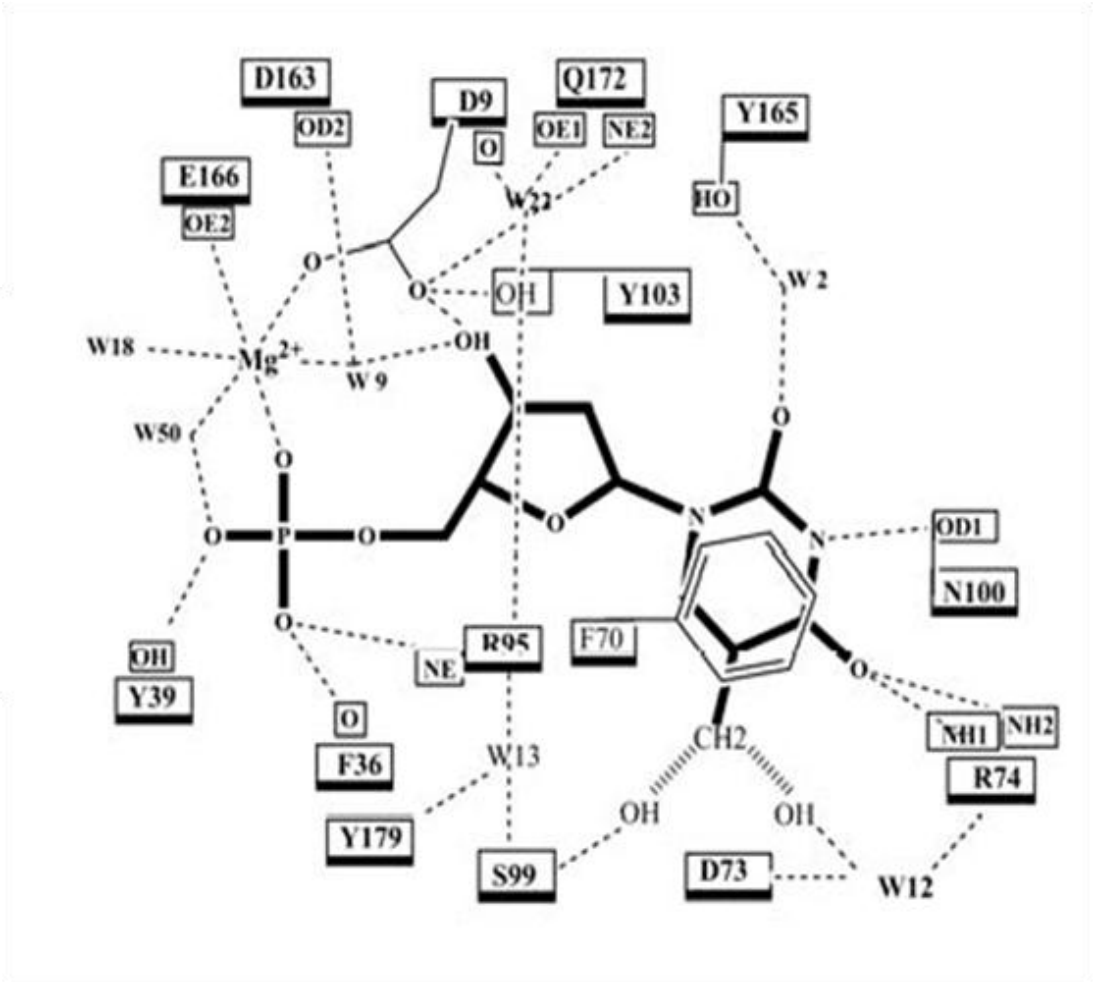


Figure 1.10. Network of interactions of **21** in the active site of TMPK*mt*.[153]

Prompted by the ability of 5-CH_2OH group to convert a substrate (dTMP) to an inhibitor and encouraged by recent modelling studies that attributed a pivotal role to this 5-CH_2OH group in TMPK*mt* binding,[154] we decided to investigate possibilities of combining this moiety with 5′-substitutions (See Chapter 6).

CHAPTER – 2

AIM AND RATIONALE OF THIS STUDY

AIM AND RATIONALE OF THIS STUDY

Modified nucleosides and nucleotides have proven to be therapeutically useful agents. They form a substantial core of the clinician's armamentarium against cancer and viral infections. Most nucleosidic anticancer drugs act by inhibiting DNA synthesis by some means. Some target DNA synthesis directly, such as the nucleosides cladribine, fludarabine and cytarabine. The triphosphate form of these drugs is incorporated into the growing DNA and acts as a chain terminator, but probably also interferes with other biochemical targets, such as ribonucleotide reductase. Others impede the supply of monomers for DNA biosynthesis and so arrest cell division (*e.g.*, the antimetabolites fluorouracil, 6-thiopurine and 6-mercaptoguanine).

The antiviral nucleoside and nucleotide drugs act as prodrugs, which after activation to the corresponding triphosphate will interfere with virus-encoded reverse transcriptase (RT) or DNA or RNA polymerase enzymes.

Several nucleoside analogues, which act on different, less established targets, are in clinical development. During the past 30 years, a number of selective agonists for adenosine receptors, all structurally derived from adenosine, have been developed. Several such compounds are undergoing or have undergone clinical trials for the treatment of cardiovascular diseases (A_1 and A_{2A}), pain (A_1), wound healing (A_{2A}), diabetic foot ulcers (A_{2A}), colorectal cancer (A_3) and rheumatoid arthritis (A_3).

The main goals of this study are:

- to improve the synthesis of apionucleosides;
- to construct apio and other nucleosides with potential pharmacological activity based on known SAR data;
- to assess the biological activity of these nucleosides in collaboration with other research groups.

Based on the discovery that the nucleoside phosphonates PMDTA and PMDTT act as potent HIV RT inhibitors[41] and on the observation that threose nucleic acids (TNA) are capable of complementary base pairing with DNA and RNA,[155] we will

investigate the antiviral activity of a series of α-L-2′-deoxythreonucleosides **Ia** (Figure 2.1). It is noteworthy that a *conditio sine qua non* for nucleosides **Ia** to act as RT inhibitors/false substrates is their efficient activation by kinases. Since, to the best of our knowledge, nucleoside kinase mediated phosphorylation of secondary OH groups is unprecedented, we will convert nucleosides **Ia** to their monophosphate prodrugs **Ib**.

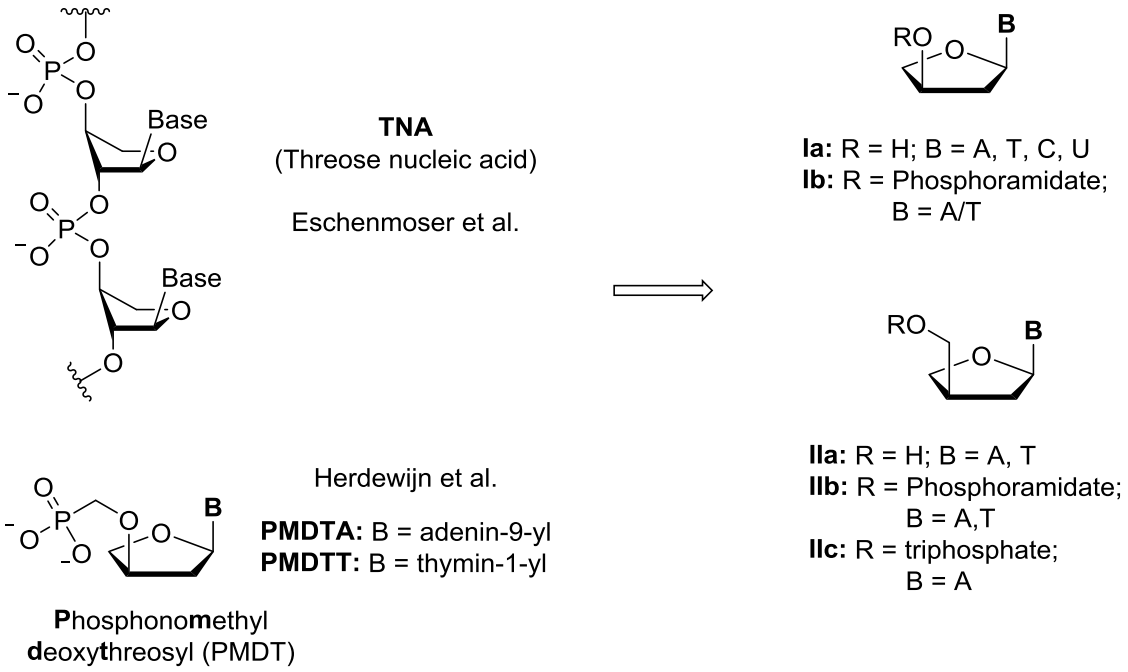


Figure 2.1. Antiviral compounds in this study.

Even though the 2′,3′-dideoxy-β-D-apio-D-furanonucleosides (ddAN) **1.7**, as well as their L-enantiomers **1.6** were reported to be inactive against HIV (Figure 2.2), we will revisit the synthesis and antiviral evaluation of these compounds, based on the observation that:

- both enantiomers of dideoxyisonucleosides (**1.71** and **1.76**), structural isomers of the envisaged ddANs, and PMDT analogues (A,T), isosteres of the monophosphate forms of the proposed ddANs, are potent HIV RT inhibitors;
- the triphosphate form of the 3′-deoxyANs **1.21**, the phosphonomethoxythreosyl phosphonate nucleoside diphosphates (**1.49**) and the isonucleoside triphosphates (**1.87**) are readily accepted by several polymerases.

If our studies would confirm that nucleosides **IIa** (= **1.7**) fail to show antiviral activity, we will synthesize the corresponding phosphate prodrugs (ProTides **IIb**) in an effort to bypass the first phosphorylation step.

To further expand our understanding of the influence of the 2′,3′-dideoxy-β-D-apio-D-furanose modified nucleosides on the orthogonal conformational requirements posed by kinases and viral polymerases, we also plan to transform a ddAN to its corresponding triphosphate (**IIc**) to test the effect of the latter on HIV RT.

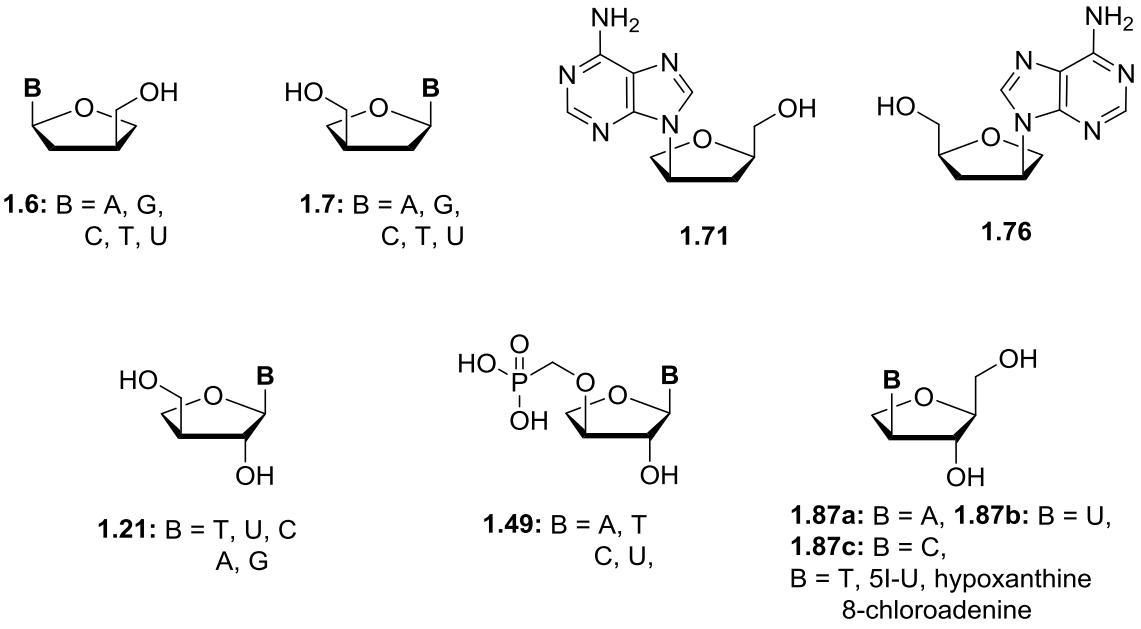


Figure 2.2. Reported antiviral compounds and compounds that are accepted readily by polymerases.

Literature precedents on the synthesis and evaluation of apionucleoside derivatives as A_3AR modulators are scarce, the recently reported carbocyclic analogue **IIIa**, a weak A_3AR agonist, being a notable exception (Figure 2.3; **IIIa**). Hence, we plan to use the synthetic methodology developed for the synthesis of the aforementioned apionucleosides to construct suitably modified ANs as possible A_3AR modulators. Modifications known to favorably influence A_3AR modulation (*e.g.*, N^6-aralkyl and 4′-carboxamide/carbamate modifications, see Chapter 1, section 1.4) will be introduced in the apioadenosine structure (Figure 2.3; **IIIb-d**).

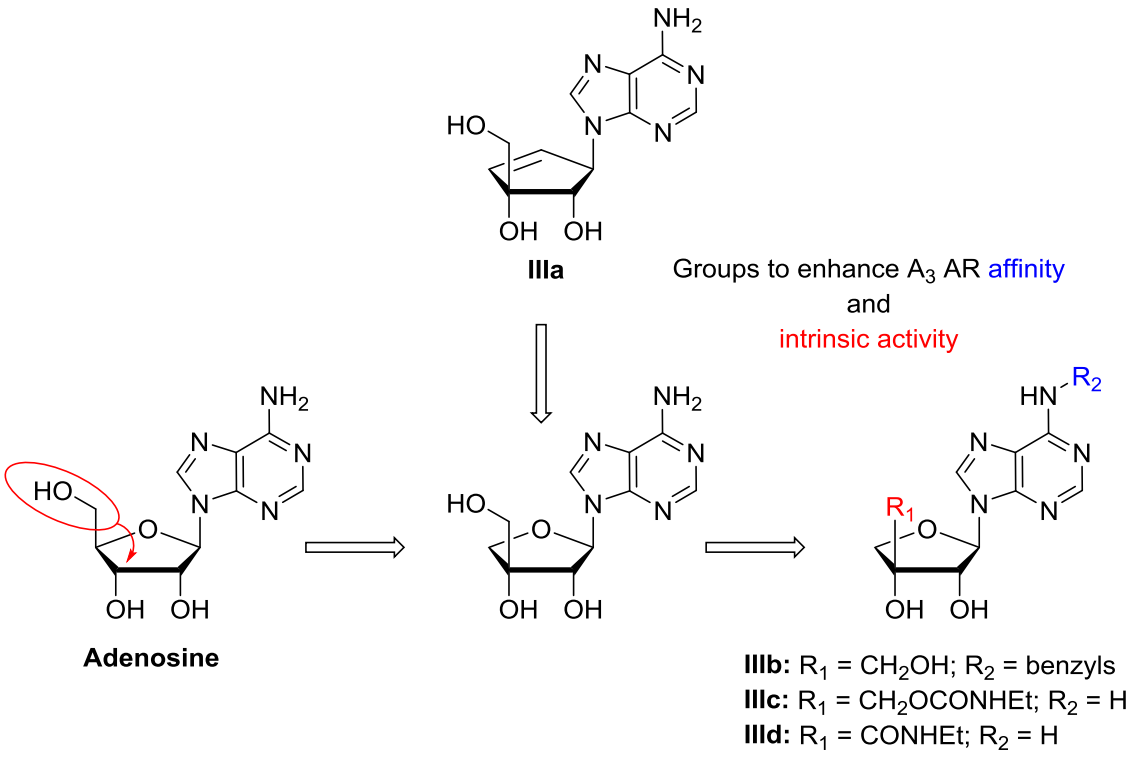


Figure 2.3. A_3AR ligands in this study.

Recently, thymidine monophosphate kinase (TMPK) has been validated as a viable target for antibacterial drug development. TMPK is an essential enzyme to convert dTMP into dTDP in the *de novo* and *salvage* DNA biosynthetic pathways. Differences in the catalytic site of *M. tuberculosis* TMPK (TMPK*mt*) compared to the human isozyme have been successfully explored to design selective inhibitors of the former. Based on the reported bicyclic TMPK*mt* inhibitors and other inhibitory data reported by our group, Frecer *et al.* recently applied combinatorial design and structure-based *in silico* screening of a virtual focused library of bicyclic thymidine analogs in an effort to identify more potent TMPK*mt* inhibitors.[154] In their study they used the three-dimensional structure of TMPK*mt* complexed with 5-hydroxymethyl-dUMP to develop a QSAR model, to parameterize a target-specific scoring function for TMPK*mt* and to select virtual hits that display the highest predicted binding to the target. Compound **IV** emerged as one of the best analogues (Figure 2.4). Also a number of *in silico* favorable 5′-substituents (**a**, **b**, and **d**) arose from this study. Since the 5-CH_2OH and 5′-substitutions were described crucial, we aim to prepare a small

series of 5′-substituted thymidines with and without 5-methyl modifications (**VI** and **Va-e**, respectively). In these target analogues the carbocyclic ring, which is mainly expected to enhance metabolic stability and the 2′,3′-bicyclic ring proposed in **IV**, are omitted to increase the synthetic feasibility.

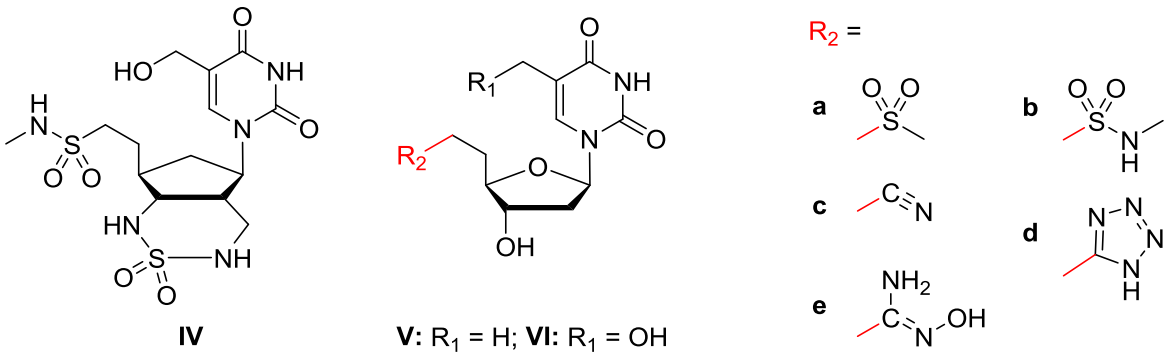


Figure 2.4. *In silico hit* **IV** and envisaged analogues **V** and **VI** as potential inhibitors of TMPK*mt*.

CHAPTER – 3

2'-DEOXYTHREOFURANOSYL NUCLEOSIDES

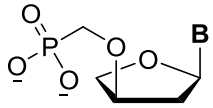
This Chapter was published as

Synthesis and antiviral evaluation of α-L-2′-deoxythreofuranosyl nucleosides. Kiran S. Toti, Marco Derudas, Christopher McGuigan, Jan Balzarini and Serge Van Calenbergh* *European Journal of Medicinal Chemistry,* **2011**, *46,* 3704-3713.

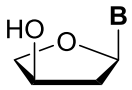
3.1. Objectives

Herdewijn and coworkers showed that selected L-2′-deoxythreose nucleoside phosphonates (Figure 3.1) selectively inhibit HIV without affecting human DNA synthesis. PMDTA and PMDTT lack a hydroxymethyl group at the 4′-position of the furanose ring, but instead have a phosphonomethyl ether moiety at the 3′-position, which mimics the monophosphate ester of a 3′-hydroxymethyl substituent. Interestingly, Eschenmoser and coworkers showed that α-L- threofuranosyl oligonucleotides (TNAs) containing vicinally connected (3′→2′) phosphodiester bridges undergo informational base pairing in antiparallel strand orientation and are capable of cross-pairing with RNA and DNA.[155]

Inspired by these findings, we decided to explore the synthesis and the antiviral properties of the corresponding non-phosphonylated 2-deoxythreose-based nucleoside analogues **3.25**-**29**, characterized by a four-carbon-only carbohydrate part. We expected that due to the absence of the 4′-hydroxymethyl group, the secondary 3′-OH could become accessible for phosphorylation by cellular nucleoside kinases and anticipated **3.25**-**29** to have a superior bioavailability compared to the phosphonates. Remarkably, the synthesis of such α-L-2′-deoxythreofuranosyl analogues (exhibiting the 1′*R*, 3′*R* configuration) has not been reported before. Moreover, the thymine congener was converted to phosphoramidate prodrug to bypass the first phosphorylation step.



PMDTA: B = adenin-9-yl
PMDTT: B = thymin-1-yl



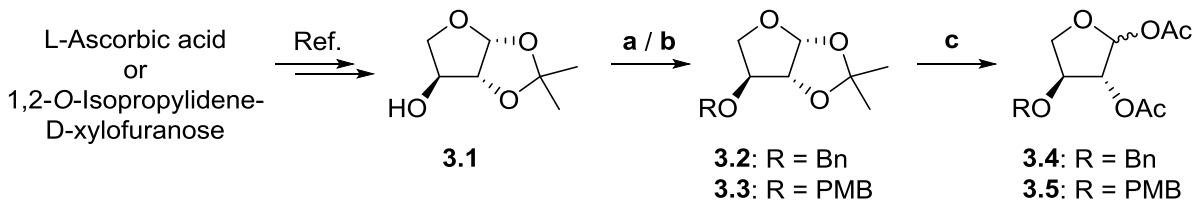
3.25: B = thymin-1-yl
3.26: B = uracil-1-yl
3.27: B = adenin-9-yl
3.29: B = cytosin-1-yl

Figure 3.1. Structures of active α-L-2′-deoxythreose nucleoside phosphonates and the nucleoside surrogates of this study (**3.25**-**27** and **3.29**).

3.2. Results and Discussion

3.2.1. Chemistry

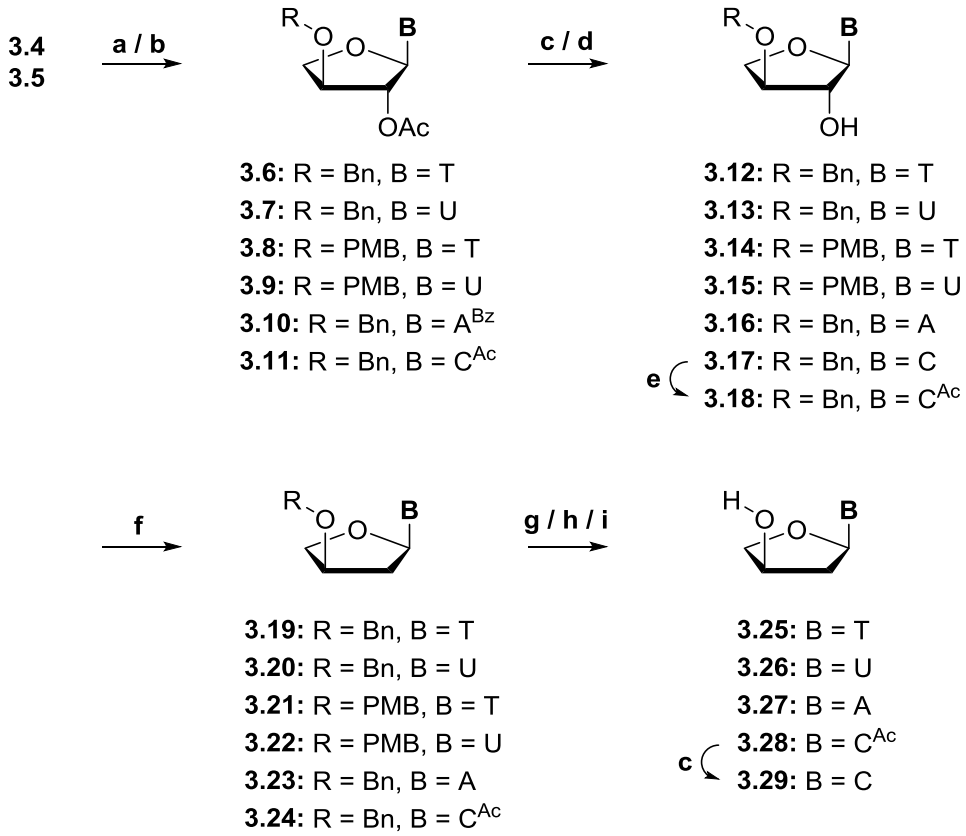
The synthesis of target compounds started from 1,2-*O*-isopropylidene-α-L-threose **3.1** (Scheme 3.1), which was synthesized in multi-gram scale following literature procedure.[156] The hydroxyl group of **3.1** was protected using either benzyl bromide or 4-methoxybenzyl (PMB) chloride to give compounds **3.2** and **3.3** in excellent yields. A benzyl group is a safe and versatile protecting group for carbohydrates, but may require harsh deprotection conditions. We also explored a more sensitive PMB protecting group, which may be removed under alternative conditions. Next, deprotection of the isopropylidene group upon treatment with 80% aq. acetic acid followed by acetylation afforded the key precursors **3.4** and **3.5** in good yield.



Scheme 3.1. Synthesis of key intermediate **3.4** and **3.5**. *Reagents and conditions:* (a) benzyl bromide, NaH, DMF, 0 °C → rt, 4h, 87%; (b) *p*-methoxybenzyl chloride, NaH, DMF, -20 °C → rt, 4h, 84%; (c) (i) 80% aq. CH_3COOH, 80 °C, 8h; (ii) Ac_2O, pyridine, rt, 4h, 63-81%.

A Vorbrüggen coupling reaction of **3.4** and **3.5** with the preformed silyl-protected nucleobases exclusively gave the α-nucleosides **3.6-11** due to anchimeric assistance from the 2-*O*-acetyl group (Scheme 3.2).[157] The benzyl-protected thymine- and uracil-nucleosides **3.6** and **3.7** were isolated in considerably better yields than the PMB protected analogues, due to instability of the PMB-protecting group under Vorbrüggen conditions. Moreover, the reaction between PMB-protected glycon **3.5** with silylated N^6-benzoyladenine and N^4-acetylcytosine failed to produce the desired coupling products in acceptable yields, which forced us to restrict further investigations to the benzyl-protected nucleosides. Under normal coupling conditions, the yield of the adenine coupling product **3.10** was also very low, possibly due to the formation of

other isomers.[158] However, prolonged reaction time at elevated temperature (50 °C) afforded the thermodynamically favored isomer **3.10** in an acceptable yield of 42%. Under the normal coupling conditions the cytidine analogue **3.11** was obtained in 70 % yield.

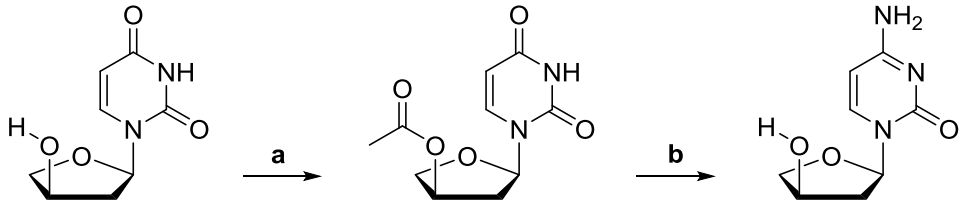


Scheme 3.2. Synthesis of α-L-2′-deoxythreofuranosyl nucleoside analogues **3.25-27** and **3.29**. *Reagents and conditions:* (a) (i) nucleobase, pyridine, TMSCl, HMDS, 135 °C, overnight; (ii) silylated base, **3.4/5**, TMSOTf, 1,2-dichloroethane, 0 °C → rt, 3-5h, 45-87%; (b) (i) nucleobase, pyridine, TMSCl, HMDS, 135 °C, overnight; (ii) silylated base, **3.4/5**, TMSOTf, 1,2-dichloroethane, 50 °C, 24h, 42%, for **3.10**; (c) 7N NH_3-MeOH, rt, 4-48h, 81-95%; (d) 1N NH_3-MeOH, rt, 5h, 78-90%, for **3.14-15**; (e) Ac_2O, DMF, rt, 24h, 78%; (f) (i) DMAP, *O-p*-tolyl chlorothionoformate, CH_3CN, 0 °C → rt, 4h; (ii) Bu_3SnH, AIBN, toluene, 100 °C, 2-4h, 47-86%; (g) Pd-C/H_2, MeOH, rt, 3-8h, 40-89%; (h) on **3.21** and **3.22,** CAN, CH_3CN:H_2O, 0 °C → rt, 1h, 78-84%; (i) DDQ, CH_2Cl_2, 50 °C, 72h, 11%, for **3.28.**

The 2′-*O*-acetyl group of **3.6** and **3.7** was removed by treatment with 7N ammonia in MeOH. Similar treatment of **3.8** and **3.9** led to the formation of small amounts of 2′,3′-dihydroxy analogues (10-15%). Lowering the ammonia concentration to 1N allowed

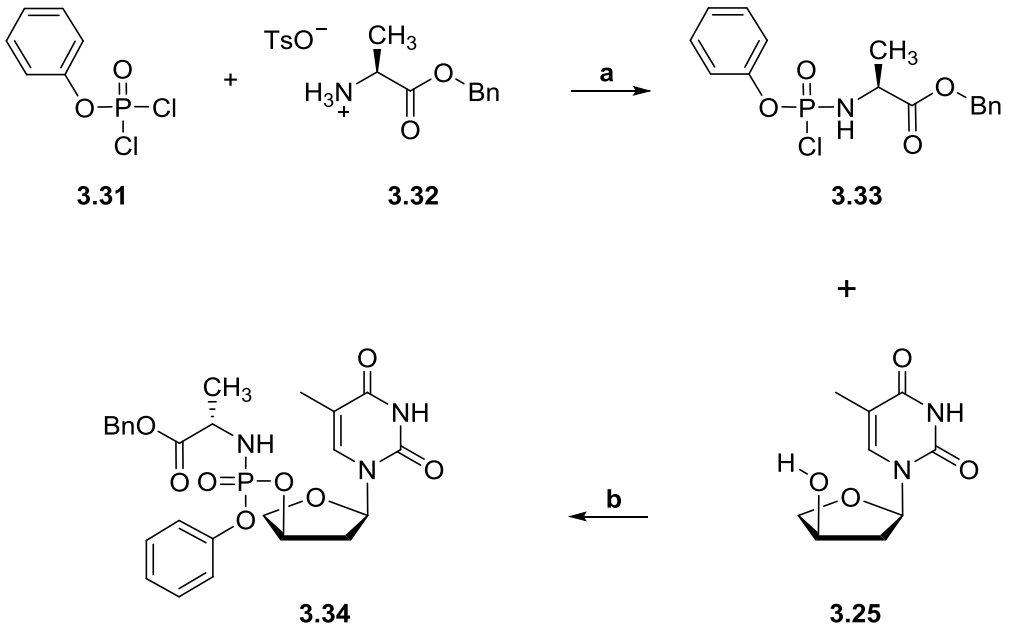
obtaining the desired compounds **3.14** and **3.15** in high yields. Stirring compound **3.10** at room temperature in a 7N solution of ammonia in MeOH for 2 days removed both the 2′-*O*-acetyl and the N^6-benzoyl group to yield **3.16**. Likewise, **3.11** was deprotected to the dideacetylated compound **3.17**, which was selectively *N*-acylated to **3.18** with one equivalent of acetic anhydride in dimethylformamide (DMF). [159] Deoxygenation of the 2′-hydroxy group was realized following a two-step Barton-McCombie procedure.[160] The xanthate was formed by reacting the 2′-OH group of **3.12-16** and **3.18** with *O*-*p*-tolyl chlorothionocarbonate in the presence of 4-dimethylaminopyridine (DMAP). The intermediate formed was subjected to heating with tributyltinhydride and azoisobisbutyronitrile in toluene to give the 2′-deoxygenated compounds **3.19-24** in moderate to good yields (47-86%).

All target compounds were obtained after debenzylation. However, optimal debenzylation procedures were distinct for each analogue. The 2′-deoxythreose thymine and uracil analogues **3.25** and **3.26** could be obtained by catalytic hydrogenation of **3.19** and **3.20** in good albeit variable yields, possibly due to catalyst poisoning by the residual sulphur from the previous deoxygenation reaction. Desulphurization with hydrogen over Raney-Nickel prior to palladium-catalyzed debenzylation improved the reproducibility of this step. Alternatively, CAN-mediated deprotection of **3.21** and **3.22** afforded **3.25** and **3.26**, respectively.[161] The adenine analogue **3.27** was obtained by a palladium-catalyzed hydrogenation reaction in moderate yields.



Scheme 3.3. An improved synthesis of the cytosine analogue **3.29**. *Reagents and conditions:* (a) acetic anhydride, DMAP, DMF, rt, 24h, 97%; (b) (i) 1,2,4-triazole, $POCl_3$, pyridine, rt, 4h; (ii) NH_3, 2h; (iii) NH_3-MeOH, rt, 5h, 36%.

The cytosine analogue **3.29** was obtained via two different routes. After an unsuccessful attempt to hydrogenate **3.24**, the benzyl group was removed by treatment with excess of 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane (CH_2Cl_2) at 50 °C in a sealed reaction vial for 3 days.[162] This method gave **3.28** in a disappointing yield of 11%. Hence, we also explored the transformation of the uridine analogue **3.26** to compound **3.29** (Scheme 3.3).[163] This was realized by protecting the 3′-hydroxy group of **3.26** to the corresponding acetate **3.30**, which was subsequently treated with phosphorous oxychloride and 1,2,4-triazole, bubbling ammonia gas and a 7N solution of ammonia in MeOH.



Scheme 3.4. Synthesis of the ProTide of **3.25**. *Reagents and conditions:* (a) anh. triethylamine (TEA), anh. CH_2Cl_2, -78 °C for 30 min, rt, 2h; (b) 1.0M *tert*-butylmagnesium chloride in THF, anh. THF, rt, 24h, 33%.

The coupling between the phenyldichlorophosphate (**3.31**) and L-alanine benzyl ester tosylate (**3.32**) has been performed in the presence of Et_3N (2 eq) giving the desired product (**3.33**) as an oil, which was used in the following step as a crude (Scheme 3.4). The final coupling of the nucleoside **3.25** has been performed using an excess of the phosphorochloridate (**3.33**) (3 eq) in the presence of *t*-BuMgCl (3eq) following the procedure reported by Uchiyama[164] and extensively used for the synthesis of the

ProTides.[165] The desired compound **3.34** was obtained as a mixture of two diastereoisomers confirmed by the presence of two peaks in the ^{31}P NMR (δ 2.92, 2.27).

3.2.2. Pharmacological evaluations

3.2.2.1. Antiviral properties

None of the final nucleoside analogues showed cytotoxicity at 90 µM. No anti-HIV-1 and anti-HIV-2 activity was observed in human T-lymphocyte (CEM) cells (EC_{50} >90 µM). Furthermore, no significant activity could be detected against the following viruses: human Cytomegalovirus (HEL), Varicella-zoster virus (VZV) (HEL); Influenza A (H1N1 and H3N2) and B virus (MDCK), Feline Corona and Feline Herpes virus (CRFK); Herpes simplex virus type 1 (HSV-1), HSV-2, VSV and Vaccinia virus (HEL); Coxsackie virus B4 and Respiratory syncytial virus (HeLa); Para-Influenza virus-3, Reovirus-3, Sindbis virus and Punta Toro virus (Vero). Since the lack of antiviral activity could be due to the inability of virally-induced and/or cellular enzymes to activate the nucleoside analogues, a nucleoside monophosphate prodrug was prepared for compound **3.25**. Such prodrugs may intracellularly release the free monophosphorylated form according to a multistep process lined-out in Scheme **3.5**. However, also the phosphoramidate **3.34** failed to show activity against HIV-1, HIV-2, HSV-1, HSV-2, Vaccinia virus, VSV and HSV-1 (TK^-).

3.2.2.2. Interaction of test compounds with nucleoside kinases

Interestingly, upon evaluation of their capacity to inhibit thymidine (1 µM) phosphorylation by recombinant purified human cytosolic TK1, human mitochondrial TK2, HSV-1 TK, VZV TK, and Drosophila melanogaster (Dm) dNK (Table 3.1), compound **3.25** proved inhibitory to TK-2, HSV-1 TK, and VZV TK at an IC_{50} ranging between 66 and 429 µM and M. tuberculosis thymidylate kinase with a K_i of 18 µM.

Table 3.1. Nucleoside kinase activity (IC_{50} in µM)

	TK-1	***TK-2***	***HSV-1 TK***	***VZV TK***	***Dm dNK***
3.25	*>942*	*429 ± 189*	*66 ± 14*	*123 ± 42*	*≥ 942*
3.26	*>1000*	*303 ± 207*	*>1000*	*>1000*	*>1000*
3.27	*>900*	*>900*	*>900*	*>900*	*>900*
3.29	*>1000*	*>1000*	*>1000*	*>1000*	*>1000*

Therefore, compound **3.25** was investigated more in detail. Whereas 75% of the natural substrate thymidine (100 µM) was converted to its 5′-mono- (and 5′-di) phosphate within 5 minutes upon exposure to HSV-1 TK, no signs of any conversion of compound **3.25** to its phosphorylated derivative was observed after 60 minutes of incubation with the enzyme. Therefore, we may assume that these nucleosides purely act as enzyme inhibitors, rather than acting as alternative substrates. Lineweaver-Burk kinetic analysis revealed a non-competitive mechanism of inhibitory action of compound **3.25** against HSV-1 TK using dThd as the natural substrate (Figure 3.2). The inhibitory potential of some of the test compounds opens perspectives for using this α-L-2′-deoxythreofuranosyl ring as a scaffold for optimizing these enzyme inhibitory activities.

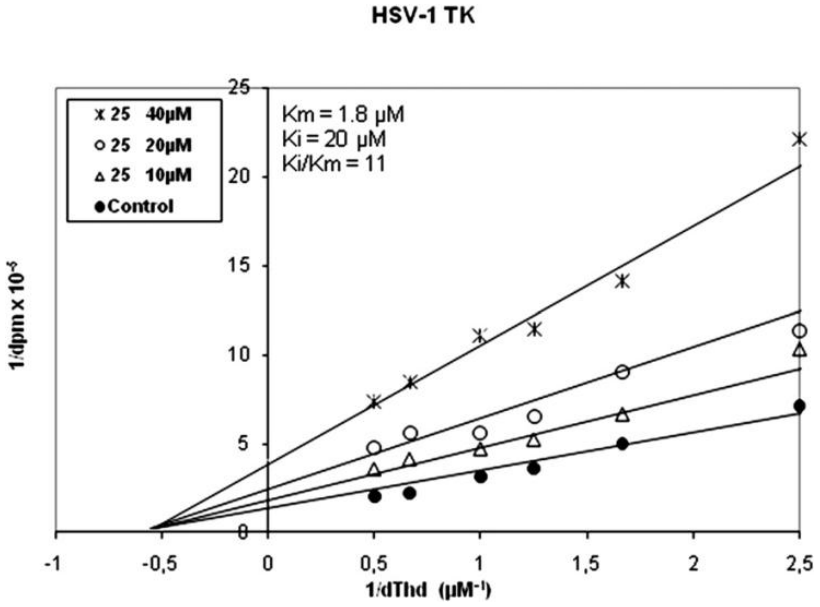
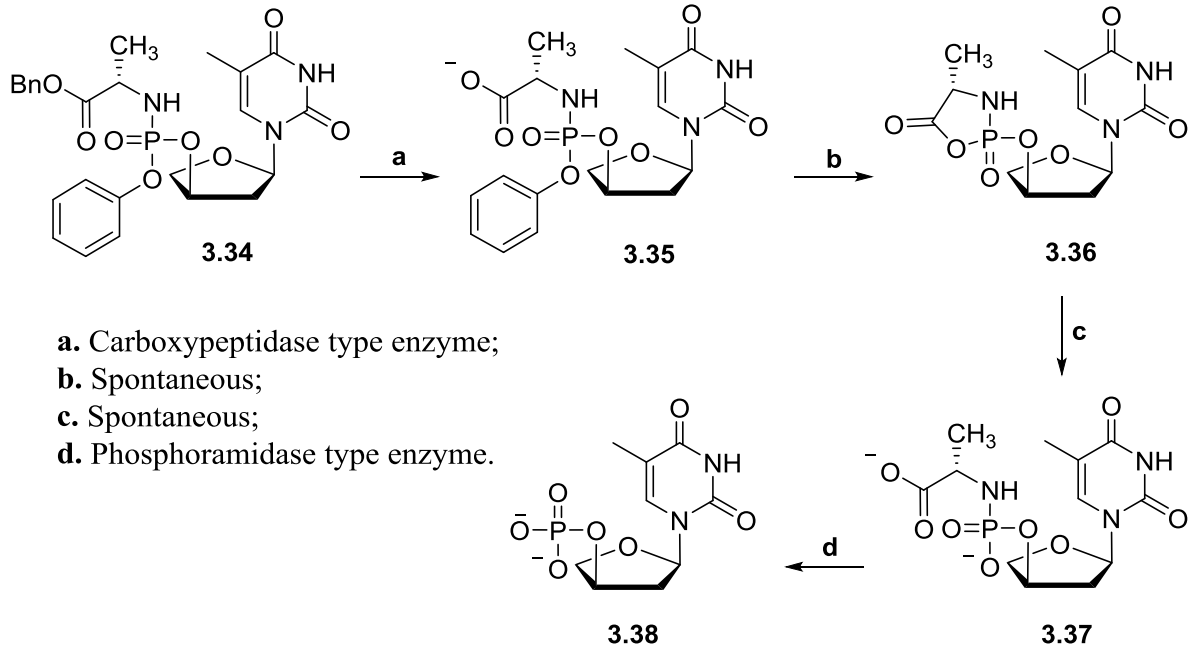


Figure 3.2. Lineweaver-Burk plot for compound **3.25**.

3.2.2.3. Enzymatic study using carboxypeptidase Y enzyme

The first step of the bioactivation of the phosphoramidate ProTide moiety involves the hydrolysis of the ester moiety which is supposed to be mediated by a carboxypeptidase type enzyme (Scheme 3.5). An enzymatic study using carboxypeptidase Y enzyme has been performed in order to understand if compound **3.34** may be metabolized under these conditions. The experiment was performed by incubating compound **3.34** with carboxypeptidase in acetone-d6 and trizma buffer (pH = 7.6) following its conversion by ^{31}P NMR. The spectra (Figure 3.3) showed a fast conversion of one of the diasteroisomers **3.34** (δP = 2.38) to the compound **3.37** (δP = 6.25) through the intermediate **3.35** (δP = 3.66). The other diastereoisomer (δP = 2.24) was slower converted and it was still detectable after 14 h. This experiment showed that **3.34** is partially converted to the metabolite **3.37**. The last step of the phosphoramidate bioactivation pathway involves the cleavage of the P-N bond in **3.37**, which is considered to be mediated by a phosphoramidase type enzyme called human Hint-1 enzyme.[166] The poor activity found for phosphoramidate prodrug **3.34** may be due to the inefficient bio-activation to the monophosphate and/ triphosphate form.



Scheme 3.5. Putative mechanism of bioactivation of the ProTide

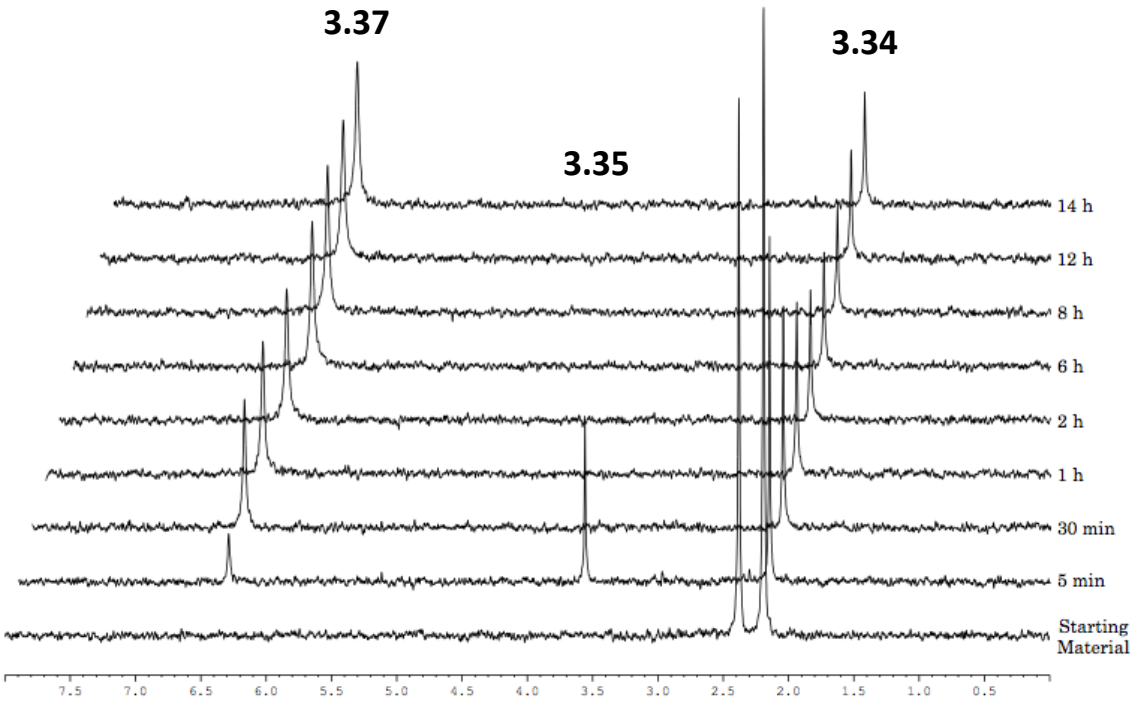


Figure 3.3. Carboxypeptidase-mediated cleavage of **3.34**, monitorated by ^{31}P NMR.

3.3. Conclusions

In conclusion, we have developed a practical and straightforward procedure for the synthesis of a series of α-L-2′-deoxythreofuranosyl nucleosides containing four natural nucleobases. None of the analogues displayed significant antiviral activity or cytotoxicity. In an effort to bypass the often problematic conversion to the monophosphate a ProTide version of thymine analogue was synthesized, which also proved to be inactive. On the other hand, the thymine analogue showed encouraging inhibitory activity towards a number of thymidine kinases, which may be a new starting point towards more potent and selective inhibitors.

3.4. Experimental Section

3.4.1. Chemical synthesis

All reagents were from standard commercial sources and of analytic grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. Moisture sensitive reactions were carried out under argon atmosphere. Precoated Merck silica gel F254 plates were used for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (63-200 μm, 60 Å, Biosolve, Valkenswaard, The Netherlands). NMR spectra were determined using a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals or TMS as internal standard. Exact mass measurements were performed on a Waters LCT PremierXETMTime of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1) mixture at 10 μL/min.

1,2-*O*-Isopropylidene-3-benzyl-β-L-threofuranose (3.2): To a solution of compound **3.1** (2.3 g, 14.36 mmol) in dry DMF (50 mL) cooled at 0 °C under inert atmosphere, NaH (60% in mineral oil, 0.861 g, 21.54 mmol) was added portionwise and the mixture was stirred for 10 minutes. Benzyl bromide (2.56 mL, 21.54 mmol) was added dropwise to the above suspension, which was allowed to come to room temperature and stirred for an additional 4h. The reaction mixture was quenched by addition of MeOH (1 mL) and the volatile solvents were evaporated under reduced pressure. The residue was partitioned between water-EtOAc (1:3, 300 mL). The organic layer was separated, washed with water, brine and dried over anhydrous $MgSO_4$. After evaporation, the crude product was purified by silica-gel chromatography (15% EtOAc-hexanes) to afford compound **3.2** (3.1 g, 87%) as a colorless oil. ^{1}H NMR($CDCl_3$, 300 MHz) δ ppm 1.32 (s, 3H), 1.47 (s, 3H), 4.01-4.05 (m, 3H), 4.57 (s, 2H), 4.61 (d, *J* = 3.77 Hz, 1H), 5.96 (d, *J* = 3.76 Hz, 1H), 7.27-7.39 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 26.15, 26.71, 70.27, 71.09, 81.81, 82.84,

105.44, 111.46, 127.59, 127.80, 128.41, 137.32. ESI-HRMS for $[C_{14}H_{18}O_4 + NH_4]^+$ calcd, 268.1549; found, 268.1565.

1,2-*O*-Isopropylidene-3-(*p*-methoxybenzyl)-β-L-threofuranose (3.3): Compound **3.1** (1.5 g, 9.4 mmol) was reacted with NaH (60% in mineral oil, 0.45 g, 11.24 mmol) and *p*-methoxybenzyl chloride (1.5 mL, 11.24 mmol) as described for synthesis of **3.2** to afford compound **3.3** (2.2 g, 84%) as a white low melting solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.31 (s, 3H), 1.46 (s, 3H), 3.78 (s, 3H), 3.98-4.02 (m, 3H), 4.48 (s, 3H), 4.58 (d, *J* = 3.76 Hz, 1H), 5.94 (d, *J* = 3.76 Hz, 1H), 6.87 (d, *J* = 8.72 Hz, 2H), 7.24 (d, *J* = 8.77 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 26.15, 26.71, 55.16, 70.28, 70.78, 81.45, 82.87, 105.42, 111.42, 113.82, 129.27, 129.34, 159, 30. ESI-HRMS for $[C_{15}H_{20}O_5 + K]^+$ calcd, 319.0942; found, 319.0937.

1,2-*O*-Diacetyl-3-benzyl-α/β-L-threofuranose (3.4): A solution of **3.2** (2.0 g, 8 mmol) in 80% aq. acetic acid (40 mL) was stirred at 80 °C for 8h. The reaction mixture was evaporated to give the crude intermediate as syrup. This syrup was dissolved in pyridine (30 mL) and treated with acetic anhydride (7.5 mL, 80 mmol). The solution was stirred at room temperature for 4h. The solvent was removed under vacuum and the resulting residue was purified by silica-gel column chromatography (20% EtOAc-hexanes) to yield **3.4** (1.9 g, 81%) as a low melting solid. α,β anomeric ratio 3:2. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.98, 2.00, 2.02, 2.03 (s's, 6H), 3.78-4.29 (m, 3H), 4.43-4.71 (m, 2H), 5.13-5.19 (m, 1H), 6.06-6.32 (s & d, *J* = 4.51 Hz, 1H), 7.20-7.32 (m, 5H). ESI-HRMS for $[C_{15}H_{18}O_6 + K]^+$ calcd, 333.0735; found, 333.0738.

1,2-*O*-Diacetyl-3-(*p*-methoxybenzyl)-α/β-L-threofuranose (3.5): Employing the procedure described above on 2.2 g of compound **3.3** resulted in 1.6 g (63% yield) of compound **3.5** as a white solid. α,β anomeric ratio 1:1. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.04, 2.07, 2.08, 2.09 (s's, 6H), 3.79 (s, 3H), 3.82-4.32 (m, 3H), 4.42-4.68 (m, 2H), 5.17-5.24 (m, 1H), 6.11, 6.37 (2d's, *J* = 4.49, 2.39 Hz, 1H), 6.83-6.91 (m, 2H), 7.20-7.30 (m, 2H). ESI-HRMS for $[C_{16}H_{20}O_7 + Na]^+$ calcd, 347.1101; found, 347.1112.

General condition for Vorbrüggen coupling reaction: The nucleobase (protected in case of adenine and cytosine) (1.2 eq.) was suspended in hexamethyldisilazane (50 eq.) containing trimethylsilyl chloride (0.7 eq.) and pyridine (10 eq.). The mixture was heated at reflux overnight. After cooling, the reaction mixture was evaporated and dried under high vacuum. The silylated nucleobase and the diacetate compound **3.4** or **3.5** (1 eq.) were dissolved in dry 1,2-dichloroethane (7 mL/mmol), and trimethylsilyl triflate (1.2 eq) was added dropwise at 0 °C. The clear solution was stirred for 4h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL/ mmol) and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. Purification of the residue by silica-gel column chromatography (1% MeOH- CH_2Cl_2) afforded the pure coupling product as white foam.

1′-(Thymin-1-yl)-2′-*O*-acetyl-3′-*O*-benzyl-α-L-threofuranose (3.6): Following the general reaction conditions a Vorbrüggen coupling between **3.4** and thymine afforded the title compound (1.07 g, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.72 (d, *J* = 1.19 Hz, 3H), 2.12 (s, 3H), 4.00-4.1 (m, 2H), 4.32 (app- dm, *J* = 8.75 Hz, 1H), 4.63 (app-dd, *J* = 14.68, 11.48 Hz, 2H), 5.22 (s, 1H), 6.08 (d, *J* = 1.36 Hz, 1H), 7.22-7.34 (m, 5H), 7.37 (d, *J* = 1.22 Hz, 1H), 9.42 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 12.57, 21.03, 71.81, 73.74, 79.93, 80.88, 89.41, 110.94, 128.17, 128.47, 128.83, 136.41, 136.89, 150.69, 164.23, 169.90. ESI-HRMS for $[C_{18}H_{20}N_2O_6 + H]^+$ calcd, 361.1400; found, 361.1399.

1′-(Uracil-1-yl)-2′-*O*-acetyl-3′-*O*-benzyl-α-L-threofuranose (3.7): Vorbrüggen coupling between **3.4** (500 mg, 1.7 mmol) and uracil (230 mg, 2.04 mmol) afforded 481 mg of the uridine analogue **3.7** (82 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.07 (s, 3H), 3.96 (d, *J* = 3.78 Hz, 1H), 4.02 (dd, *J* = 10.25, 3.81 Hz, 1H), 4.24 (d, *J* = 10.28 Hz, 1H), 4.56 (app-q, *J* = 11.76 Hz, 2H), 5.17 (s, 1H), 5.55 (dd, *J* = 8.19, 1.77 Hz, 1H), 5.96 (d, *J* = 1.18 Hz, 1H), 7.17-7.32 (m, 5H), 7.48 (d, *J* = 8.20 Hz, 1H), 8.93 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 21.03, 71.88, 74.27, 79.72, 80.52, 89.82, 102.23, 128.28, 128.54, 128.87, 136.80, 140.58, 150.39, 163.39, 169.79. ESI-HRMS for $[C_{17}H_{18}N_2O_6 + H]^+$ calcd, 347.1238; found 347.1246.

1′-(Thymin-1-yl)-2′-*O*-acetyl-3′-*O*-(*p*-methoxybenzyl)-α-L-threofuranose (3.8): Vorbrüggen coupling between **3.5** (600 mg, 1.85 mmol) and thymine yielded 420 mg of the title compound (58 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.68 (d, *J* = 1.21 Hz, 3H), 2.07 (s, 3H), 3.73 (s, 3H), 3.98 (dd, *J* = 14.17, 3.72 Hz, 2H), 4.14-4.25 (m, 1H), 4.50 (app-q, *J* = 14.14 Hz, 2H), 5.15 (m, 1H), 6.00 (d, *J* = 1.34 Hz, 1H), 6.80 (d, *J* = 8.72 Hz, 2H), 7.14 (d, *J* = 8.78 Hz, 2H), 7.31 (d, *J* = 1.24 Hz, 1H), 8.76 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 12.60, 21.05, 55.54, 71.48, 73.85, 79.87, 80.53, 89.45, 110.83, 114.21, 128.95, 129.88, 136.50, 150.48, 159.84, 163.97, 169.87. ESI-HRMS for $[C_{19}H_{22}N_2O_7 + H]^+$ calcd, 391.1500; found, 391.1521.

1′-(Uracil-1-yl)-2′-*O*-acetyl-3′-*O*-(*p*-methoxybenzyl)-α-L-threofuranose (3.9): Vorbrüggen coupling between **3.5** (325 mg, 1.0 mmol) and uracil yielded compound **3.9** (180 mg, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.12 (s, 3H), 3.79 (s, 3H), 4.00 (d, *J* = 3.73 Hz, 1H), 4.07 (dd, *J* = 10.24, 3.81 Hz, 1H), 4.27 (d, *J* = 10.23 Hz, 1H), 4.55 (app-q, *J* = 12.73 Hz, 2H), 5.23 (s, 1H), 5.62 (dd, *J* = 8.21, 1.47 Hz, 1H), 6.02 (d, *J* = 1.03 Hz, 1H), 6.86 (d, *J* = 8.81 Hz, 2H), 7.19 (d, *J* = 8.79 Hz, 2H), 7.54 (d, *J* = 8.21 Hz, 1H), 9.36 (brm, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 21.00, 55.50, 71.42, 74.32, 79.61, 80.15, 89.76, 102.16, 114.20, 128.91, 129.99, 140.68, 150.71, 159.80, 164.03, 169.85. ESI-HRMS for $[C_{18}H_{20}N_2O_7 + H]^+$ calcd, 377.1343; found 377.1369.

1′-(N^6-Benzoyladenin-9-yl)-2′-*O*-acetyl-3′-*O*-benzyl-α-L-threofuranose (3.10): To a suspension of N^6-benzoyladenine (1.0 g, 4.08 mmol) in hexamethyldisilazane (35 mL, 0.17 mol) was added trimethylsilyl chloride (0.3 mL, 2.38 mmol) and pyridine (2.7 mL, 34 mmol). The mixture was heated at reflux overnight. After cooling it was evaporated and dried under high vacuum. The silylated nucleobase and the diacetate **3.4** (1.0 g, 3.4 mmol) were dissolved in dry 1,2-dichloroethane (20 mL) and trimethylsilyl triflate (0.74 mL, 4.08 mmol) was added dropwise at 0 °C. The clear solution was stirred at 50 °C for 24h. After dilution with CH_2Cl_2 (250 mL), the reaction mixture was washed with saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. purification of the residue by silica-gel flash column chromatography (60 % EtOAc – hexanes) afforded pure **3.10** (0.68 g, 42%) as white foam.^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.08 (s, 3H), 4.04 (d, *J* = 3.76

Hz, 1H), 4.31 (dd, *J* = 10.34, 3.89 Hz, 1H), 4.4 - 4.52 (m, 3H), 5.48 (s, 1H), 6.71 (s, 1H), 7.00 - 7.10 (m, 5H), 7.31 – 7.50 (m, 3H), 8.08 (s, 1H), 8.12 – 8.19 (m, 2H), 8.59 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 21.03, 72.12, 76.07, 79.94, 80.49, 93.07, 127.81, 128.08, 128.16, 128.22, 128.56, 128.74, 129.89, 132.32, 136.84, 137.81, 142.01, 143.61, 148.71, 169.68, 175.13. ESI-HRMS for $[C_{25}H_{23}N_5O_5 + H]^+$ calcd, 474.1772; found, 474.1698.

1′-(*N*^{4}-Acetylcytosin-1-yl)-2′-*O*-acetyl-3′-*O*-benzyl-α-L-threofuranose (3.11): A Vorbrüggen coupling reaction between *N*^{4}-acetylcytosine and compound **3.4** (295 mg, 1.0 mmol) yielded compound **3.11** (270 mg, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.11 (s, 3H), 2.28 (s, 3H), 4.01 (d, *J* = 3.77 Hz, 1H), 4.20 (dd, *J* = 10.31, 3.86 Hz, 1H), 4.35 (d, *J* = 10.25 Hz, 1H), 4.97 (app-q, *J* = 13.94 Hz, 2H), 5.28 (s, 1H), 5.34 (s, 1H), 6.05 (s, 1H), 7.14 - 7.20 (m, 2H), 7.25 – 7.32 (m, 3H), 7.34 (d, *J* = 7.61 Hz, 1H), 7.89 (d, *J* = 7.57 Hz, 1H), 10.34 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 21.10, 25.10, 71.67, 75.25, 79.15, 80.15, 91.22, 96.48, 128.21, 128.37, 128.77, 136.84, 145.07, 155.25, 163.53, 169.70, 171.44. ESI-HRMS for $[C_{19}H_{21}N_3O_6 + H]^+$ calcd, 388.1503; found, 388.1509

1′-(Thymin-1-yl)-3′-*O*-benzyl-α-L-threofuranose (3.12): The 2′-*O*-acetylated nucleoside **3.6** (580 mg, 1.6 mmol) was treated with 7N methanolic ammonia solution (15 mL) at room temperature overnight. Evaporation yielded a residue which was purified by column chromatography (2% MeOH-CH_2Cl_2) to afford compound **3.12** (420 mg, 82 %) as a white foam.^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.67 (d, *J* = 0.75 Hz, 3H), 4.07 (s, 1H), 4.30 (s, 2H), 4.42 (s, 3H), 5.59 (br. s, 1H), 7.78 (s, 1H), 7.06-7.24 (m, 5H), 7.30 (d, *J* = 1.14 Hz, 1H), 10.60 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 12.24, 71.45, 74.97, 78.10, 82.20, 93.54, 109.38, 127.59, 127.96, 128.45, 136.75, 136.92, 150.96, 164.73. ESI-HRMS for $[C_{16}H_{18}N_2O_5 – H]^-$ calcd, 317.1143; found 317.1147.

1′-(Uracil-1-yl)-3′-*O*-benzyl-α-L-threofuranose (3.13): Following the procedure described for **3.12**, compound **3.7** (475 mg, 1.37 mmol) was hydrolysed to afford **3.13** (340 mg, 81%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 4.05 (s, 1H), 4.28

(s, 2H), 4.41 (s, 1H), 4.43 (s, 2H), 5.54 (dd, *J* = 8.10, 1.81 Hz, 1H), 5.73 (s, 1H), 7.10-7.30 (m, 5H), 7.48 (d, *J* = 8.16 Hz, 1H), 10.52 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm 71.85, 75.31, 82.29, 94.00, 101.32, 128.01, 128.37, 128.79, 137.11, 140.94, 151.30, 164.38. ESI-HRMS for $[C_{15}H_{16}N_2O_5 + H]^+$ calcd, 305.1132; found, 305.1132.

1′-(Thymin-1-yl)-3′-*O*-(*p*-methoxybenzyl)-α-L-threofuranose (3.14): Compound **3.8** (400 mg, 1.02 mmol) was treated with 1N methanolic ammonia solution (10 mL) at room temperature for 6h. Evaporation yielded a residue which was purified by column chromatography (2% MeOH-CH_2Cl_2) to afford compound **3.14** (280 mg, 78 %) as a white foam.^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.69 (d, *J* = 1.1 Hz, 3H), 3.71 (s, 3H), 4.03 (m, 1H), 4.27 (d, *J* = 1.84 Hz, 2H), 4.35 (s, 2H), 4.40 (s, 1H), 5.76 (s, 1H), 6.76 (d, *J* = 8.72 Hz, 2H), 7.03 (d, *J* = 8.70 Hz, 2H), 7.29 (d, *J* = 1.21 Hz, 1H),10.47 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.57, 55.54, 71.41, 75.15, 78.48, 82.24, 93.77, 109.64, 114.15, 129.30, 129.57, 137.02, 151.20, 159.67, 164.92. ESI-HRMS for $[C_{17}H_{20}N_2O_6 + H]^+$ calcd, 349.1394; found, 349.1389.

1′-(Uracil-1-yl)-3′-*O*-(*p*-methoxybenzyl)-α-L-threofuranose (3.15): The ester moiety of **3.9** (180 mg, 0.48 mmol) was hydrolysed as described in the preparation of **3.14** to give compound **3.15** (145 mg, 90%) as white foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 3.77 (s, 3H), 4.08-4.12 (m, 1H), 4.26-4.36 (m, 2H), 4.42 (s, 2H), 4.46 (s, 1H), 5.55 (br. s, 1H), 5.60 (dd, *J* = 8.14, 1.74 Hz, 1H), 5.79 (s, 1H), 6.83 (d, *J* = 8.77 Hz, 2H), 7.11 (d, *J* = 8.65 Hz, 2H), 7.53 (d, *J* = 8.19 Hz, 1H), 10.74 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 55.52, 71.50, 75.44, 78.24, 82.05, 93.93, 101.26, 114.17, 129.28, 129.69, 141.05, 151.40, 159.71, 164.51. ESI-HRMS for $[C_{16}H_{18}N_2O_6 + H]^+$ calcd, 335.1238; found, 335.1255.

1′-(Adenin-9-yl)-3′-*O*-benzyl-α-L-threofuranose (3.16): Nucleoside **3.10** (680 mg, 1.43 mmol) was treated with 7N methanolic ammonia solution (20 mL) at room temperature for 2 days. Evaporation yielded a residue which was purified by column chromatography (4% MeOH-CH_2Cl_2) to afford compound **3.16** (450 mg, 95 %) as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm 4.10 – 4.15 (m, 1H), 4.19 (dd, *J* = 9.75, 4.72 Hz, 1H), 4.29 (dd, *J* = 9.72, 2.61 Hz, 1H), 4.57 (s, 2H), 4.66 – 4.71 (m, 1H),

5.95 (d, *J* = 2.28 Hz, 1H), 6.02 (d, *J* = 4.52 Hz, 1H), 7.20 – 7.40 (m, 7H), 8.16 (s, 2H). ^{13}C NMR (DMSO- d_6, 75 MHz) δ ppm 75.82, 78.18, 82.47, 87.32, 95.55, 123.17, 131.76, 131.96, 132.43, 141.20, 143.53, 152.62, 156.47, 159.74. ESI-HRMS for $[C_{16}H_{18}N_5O_3 + H]^+$ calcd, 328.1404; found, 328.1419.

1′-(Cytosin-1-yl)-3′-*O*-benzyl-α-L-threofuranose (3.17): The 2′-*O*-acetylated nucleoside **3.11** (210 mg, 0.54 mmol) was treated with 7N methanolic ammonia solution (15 mL) at room temperature overnight. Evaporation yielded a residue which was purified by column chromatography (2% MeOH-CH_2Cl_2) to afford 140 mg of compound **3.17** (85% yield) as a white foam. ^{1}H NMR (DMSO- d_6, 300 MHz) δ ppm 3.94 (app-d, *J* = 3.98 Hz, 1H), 4.10 (dd, *J* = 10.08, 4.06 Hz, 1H), 4.18 (d, *J* = 4.28 Hz, 1H), 4.28 (d, *J* = 10.08 Hz, 1H), 4.47 (app-q, *J* = 11.70 Hz, 2H), 5.60 (d, *J* = 7.44 Hz, 1H), 5.68 (d, *J* = 1.08 Hz, 1H), 5.75 (s, 1H), 5.80 (d, *J* = 4.35 Hz, 1H), 7.00 (brd, *J* = 23.36 Hz, 2H), 7.19 – 7.26 (m, 2H), 7.26 – 7.35 (m, 3H), 7.53 (d, *J* = 7.41 Hz, 1H). ^{13}C NMR (DMSO- d_6, 75 MHz) δ ppm 55.60, 70.91, 73.60, 78.12, 83.64, 93.06, 93.53, 128.18, 128.34, 128.86, 138.44, 142.12, 155.94, 166.44. ESI-HRMS for $[C_{15}H_{17}N_3O_4 + H]^+$ calcd, 304.1292; found, 304.1294.

1′-(N^4-Acetylcytosin-1-yl)-3′-O-benzyl-α-L-threofuranose (3.18): To a solution of compound **3.17** (180 mg, 0.59 mmol) in dry DMF (1.5 mL) was added acetic anhydride (56 µL, 0.59 mmol) and the mixture was stirred at room temperature for 24h. The residue obtained after distillation of the volatiles under reduced pressure, was purified by silica-gel column chromatography to give **3.18** (160 mg, 78%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.2 (s, 3H), 4.00 – 4.04 (m, 1H), 4.23 (dd, *J* = 9.79, 1.48 Hz, 1H), 4.29 (dd, *J* = 9.93, 3.88 Hz, 1H), 4.39 (s, 1H), 4.42 (s, 1H), 4.85 (br. s, 1H), 5.73 (d, *J* = 0.75 Hz, 1H), 7.05 – 7.11 (m, 2H), 7.17 – 7.25 (m, 3H), 7.31 (d, *J* = 7.50 Hz, 1H), 7.88 (d, *J* = 7.50 Hz, 1H), 9.36 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 25.14, 71.68, 74.99, 78.65, 82.67, 94.89, 96.26, 127.96, 128.17, 128.69, 137.25, 145.33, 156.17, 163.04, 171.17, 177.08. ESI-HRMS for $[C_{17}H_{19}N_3O_5 + H]^+$ calcd, 346.1397; found, 346.1400.

Barton – McCombie deoxygenation procedure for the synthesis of compounds 3.19 – 3.24. To an ice-cold solution of compound **3.12** – **3.18** (1 eq.) and DMAP (2 eq.) in CH_3CN (25 mL/mmol) was gradually added *O*-*p*-tolylchlorothionocarbonate (1.2 equivalents) at 0 °C and stirring was continued at room temperature. After 2-4h the solvent was removed in vacuo and the residue was dissolved in EtOAc. The solution was washed twice with water, dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo to give the corresponding xanthate as yellow solid/syrup. The xanthate was dissolved in toluene (50 mL/mmol), to which azobisisobutyronitrile (AIBN, 2 equivalent) was added. Tri-*n*-butyltin hydride (2.5 equivalent) was added to this mixture at 60-70 °C, which was further stirred for 2-4h at 95-100 °C. The solvent was removed in vacuum and the residue was purified by column chromatography (30-40 % EtOAc - hexanes) to yield the 2′-deoxy compounds **3.19** – **3.24** as a foam.

1′-(Thymin-1-yl)-2′-deoxy-3′-*O*-benzyl-α-L-threofuranose (3.19): Deoxygenation of **3.12** (420 mg, 1.32 mmol) rendered 330 mg of compound **3.19** in 82% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.69 (d, *J* = 1.19 Hz, 3H), 2.19 (ddd, *J* = 15.07, 1.19, 0.88 Hz, 1H), (ddd, *J* = 15.11, 7.94, 5.45 Hz, 1H), 3.82 (dd, *J* = 10.27, 3.60 Hz, 1H), 4.19 (dd, *J* = 5.47, 3.66 Hz, 1H), 4.30 (dd, *J* = 10.33, 1.50 Hz, 1H), 4.42 (app-dd, *J* = 15.15, 11.27 Hz, 2H), 6.17 (*J* = 7.88, 2.17 Hz, 1H), 7.16-7.32 (m, 5H), 7.47 (d, *J* = 1.22 Hz, 1H), 8.67 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 12.60, 38.49, 71.41, 74.51, 77.58, 85.48, 110.35, 127.90, 128.32, 128.82, 136.98, 137.28, 150.76, 164.10. ESI-HRMS for $[C_{16}H_{18}N_2O_4 + H]^+$ calcd, 303.1339; found, 303.1342.

1′-(Uracil-1-yl)-2′-deoxy-3′-*O*-benzyl-α-L-threofuranose (3.20): Deoxygenation of **3.13** (306 mg, 1.0 mmol) rendered 250 mg of compound **3.20** in 86% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.23 (dm, *J* = 15.11 Hz, 1H), 2.39 (ddd, *J* = 15.11, 7.56, 5.31 Hz, 1H), 3.85 (dd, *J* = 10.25, 3.67 Hz, 1H), 4.17 (app-t, *J* = 4.40 Hz, 1H), 4.28 (dd, *J* = 10.24, 1.83 Hz, 1H), 4.41 (app-dd, *J* = 13.75, 11.63 Hz, 2H), 5.55 (dd, *J* = 8.16, 1.73 Hz, 1H), 6.12 (dd, *J* = 7.54, 2.00 Hz, 1H), 7.14-7.34 (m, 5H), 7.61 (d, *J* = 8.16 Hz, 1H), 8.86 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 38.56, 71.39, 74.99, 77.28, 86.15, 101.69, 127.96, 128.36, 128,85, 137.20, 141.09, 150.70, 163.63. ESI-HRMS for $[C_{15}H_{16}N_2O_4 + H]^+$ calcd, 289.1183; found, 289.1183.

1′-(Thymin-1-yl)-2′-deoxy-3′-*O*-(*p*-methoxybenzyl)-α-L-threofuranose (3.21): Deoxygenation reaction of **3.14** (280 mg, 0.81 mmol) rendered 200 mg of compound **3.21** in 75% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.70 (d, *J* = 1.20 Hz, 3H), 2.16 (ddd, *J* = 15.09, 2.04, 1.05 Hz, 1H), 2.40 (ddd, *J* = 15.1, 7.93, 5.52 Hz, 1H), 3.73 (s, 3H), 3.80 (dd, *J* = 10.22, 3.66 Hz, 1H), 4.17 (app-t, *J* = 4.46 Hz, 1H), 4.28 (dd, *J* = 10.26, 1.34 Hz, 1H), 4.35 (dd, *J* = 14.60, 11.00 Hz, 2H), 6.16 (dd, *J* = 7.90, 2.20 Hz, 1H), 6.80 (d, *J* = 8.74 Hz, 2H), 7.12 (d, *J* = 8.74 Hz, 2H), 7.46 (d, *J* = 1.23 Hz, 1H), 8.69 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 12.65, 38.44, 55.55, 71.06, 74.57, 85.50, 110.28, 114.21, 129.37, 129.56, 137.04, 150.79, 159.71, 164.16. ESI-HRMS for $[C_{17}H_{20}N_2O_5 + H]^+$ calcd, 333.1445; found, 333.1461.

1′-(Uracil-1-yl)-2′-deoxy-3′-*O*-(*p*-methoxybenzyl)-α-L-threofuranose (3.21): Deoxygenation of **3.15** (100 mg, 0.3 mmol) rendered 70 mg of compound **3.22** in 74% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.21 (app-dm, *J* = 15.15 Hz, 1H), 2.37 (ddd, *J* = 15.03, 7.47, 5.28 Hz, 1H), 3.73 (s, 3H), 3.83 (dd, *J* = 10.20, 3.66 Hz, 1H), 4.25 (dd, *J* = 10.14, 1.17 Hz, 1H), 4.33 (d, *J* = 1.40 Hz, 2H), 5.56 (d, *J* = 8.13 Hz, 1H), 6.11 (dd, *J* = 7.54, 1.96 Hz, 1H), 6.80 (d, *J* = 8.70 Hz, 2H), 7.09 (d, *J* = 8.70 Hz, 2H), 7.61 (d, *J* = 8.13 Hz, 1H), 9.21 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 38.46, 55.53, 71.00, 75.06, 76.97, 86.18, 101.67, 114.22, 129.28, 129.63, 141,24, 150.96, 159.70. ESI-HRMS for $[C_{16}H_{18}N_2O_5 + H]^+$ calcd, 319.1294; found, 319.1295.

1′-(Adenin-9-yl)-2′-deoxy-3′-*O*-benzyl-α-L-threofuranose (3.23): Deoxygenation of **3.16** (450 mg, 1.38 mmol) rendered 200 mg of compound **3.23** in 47% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm 2.59 (dm, *J* = 14.60 Hz, 1H), 2.70 (ddd, *J* = 14.60, 7.61, 5.84 Hz, 1H), 4.00 (dd, *J* = 9.88, 4.45 Hz, 1H), 4.25 (app-dt, *J* = 10.05, 1.30 Hz, 1H), 4.41 (m, 1H), 4.54 (app-d, *J* = 2.42, 2H), 6.34 (dd, *J* = 7.55, 2.64 Hz, 1H), 7.24 (br. s, 2H), 7.26 – 7.38 (m, 5H), 8.24 (s, 1H), 8.22 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm 37.32, 70.30, 73.21, 77.47, 82.78, 118.52, 127.43, 127.53, 128.18, 137.87, 138.79, 149. 04, 152. 47, 155.85. ESI-HRMS for $[C_{16}H_{17}N_5O_2 + H]^+$ calcd, 312.1455; found, 312.1469.

1′-(N^4-Acetylcytosin-1-yl)-2′-deoxy-3′-*O*-benzyl-α-L-threofuranose (3.24): Deoxygenation of **3.18** (190 mg, 0.55 mmol) rendered 100 mg of compound **3.24** in 56% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 2.20 (s, 3H), 2.35 – 2.44 (m, 2H), 3.98 (dd, *J* = 10.12, 3.72 Hz, 1H), 4.13 - 4.18 (m, 1H), 4.31 (s, 2H), 4.34 (dd, *J* = 10.20, 0.96 Hz, 1H), 6.09 (dd, *J* = 6.08, 2.31 Hz, 1H), 7.06 – 7.12 (m, 2H), 7.21 (d, *J* = 8.44 Hz, 1H), 7.20 – 7.28 (m, 3H), 7.91 (d, *J* = 7.50 Hz, 1H), 9.46 (br. s, 1H). ^{13}CNMR ($CDCl_3$, 75 MHz) δ ppm 25.06, 38.46, 71.08, 75.89, 77.20, 88.21, 96.20, 127.92, 128.19, 128.76, 137.23, 145.37, 155.54, 163.35, 171.57. ESI-HRMS for $[C_{17}H_{19}N_3O_4 + H]^+$ calcd, 330.1448; found, 330.1463.

Deprotection method – A. A solution of benzyl protected nucleoside in MeOH (40 mL/mmol) was hydrogenated at atmospheric pressure for 5-24h in the presence of 10% Pd/C (150 mg/mmol). The catalyst was removed by filtration over a Celite path and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (3% MeOH-CH_2Cl_2) to isolate the desired product as a white amorphous solid.

Deprotection method – B. To a solution of benzyl protected nucleoside (1 eq.) in an acetonitrile-water (5:1) mixture (20 mL/mmol) at 0 °C ceric ammonium nitrate (CAN, 2.5 equivalents) was added and stirring was continued for 1h. The reaction mixture was treated with solid $NaHCO_3$ (2.0 g/mmol) followed by 25% MeOH-CH_2Cl_2 (50 mL/mmol). The mixture was filtered over celite and washed with 25% MeOH-CH_2Cl_2 (100 mL/mmol). The filtrate was concentrated in vacuum, and the residue was purified by column chromatography (3% MeOH-CH_2Cl_2) to isolate the final product as a white amorphous solid.

1′-(Thymin-1-yl)-2′-deoxy-α-L-threofuranose (3.25): Employing deprotection method A, 30 mg of title compound was obtained in 75 % yield from **3.19** (57 mg, 0.19 mmol), while method B afforded 80 mg of the same compound from **3.21** (150 mg, 0.45 mmol) in 84 % yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm 1.77 (d, *J* = 1.16 Hz, 3H), 1.87 (ddd, *J* = 14.49, 2.89, 1.52 Hz, 1H), 2.46 (ddd, *J* = 14.05, 8.07, 5.59 Hz, 1H), 3.77 (dd, *J* = 9.37, 3.72 Hz, 1H), 3.98 (dt, *J* = 9.36, 1.41 Hz, 1H), 4.36

(brm, 1H), 5.29 (d, *J* = 2.68 Hz, 1H), 6.05 (dd, *J* = 8.13, 2.72 Hz, 1H), 7.76 (d, *J* = 1.21 Hz, 1H), 11.23 (br. s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm 13.06, 40.75, 69.54, 77.04, 85.32, 109.20, 137.79, 151.21, 164.54. ESI-HRMS for $[C_9H_{12}N_2O_4 - H]^-$ calcd, 211.0724; found, 211.0732.

1′-(Uracil-1-yl)-2′-deoxy-α-L-threofuranose (3.26): Employing deprotection method A, 43 mg of the title compound was obtained in 89% yield from **3.20** (70 mg, 0.24 mmol), while using method B compound **3.22** (70 mg, 0.22 mmol) was converted to 34 mg of the title compound (78 % yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm 1.87 (ddd, , *J* = 14.53, 2.91, 1.88 Hz, 1H), 2.43 (ddd, *J* = 14.45, 8.00, 5.48 Hz, 1H), 3.78 (dd, *J* = 9.43, 3.66 Hz, 1H), 3.97 (app-dt, *J* = 9.46, 1.25 Hz, 1H), 4.34 (t, *J* = 4.23 Hz, 1H), 5.27 (br. s, 1H), 5.60 (d, *J* = 8.05 Hz, 1H), 6.01 (dd, *J* = 8.00, 2.23 Hz, 1H), 7.85 (d, *J* = 8.11 Hz, 1H), 11.19 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 41.64, 70.08, 78.56, 87.97, 100.61, 141.97, 150.95, 165.38. ESI-HRMS for $[C_8H_{10}N_2O_4 + H]^+$ calcd, 199.0713; found, 199.072.

1′-(Adenin-9-yl)-2′-deoxy-α-L-threofuranose (3.27): Using method A with catalytic amount of acetic acid compound **3.23** (100 mg, 0.32 mmol) was converted to 29 mg of compound **3.27** in 40 % yield. ^{1}H NMR (DMSO-D_6, 300 MHz) δ ppm 2.29 (ddd, *J* = 14.44, 2.70, 1.54 Hz, 1H), 2.68 (ddd, *J* = 14.42, 8.24, 6.10 Hz, 1H), 3.91 (dd, *J* = 9.30, 4.10 Hz, 1H), 3.97 (ddd, *J* = 9.32, 2.10, 1.06 Hz, 1H), 4.48 (m, 1H), 5.79 (d, *J* = 4.35 Hz, 1H), 6.28 (dd, *J* = 8.27, 2.56 Hz, 1H), 7.28 (s, 2H), 8.15 (s, 1H), 8.36 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm 40.86, 69.95, 76.98, 83.83, 119.53, 140.40, 149.53, 153.06, 156.73. ESI-HRMS for $[C_9H_{11}N_5O_2 + H]^+$ calcd, 222.0986; found, 222.0938.

1′-(N^4-Acetylcytosin-1-yl)-2′-deoxy-α-L-threofuranose (3.28): DDQ (770 mg, 3.4 mmol) was added to a stirring solution of compound **3.24** (112 mg, 0.34 mmol) in dry CH_2Cl_2 (5.0 mL). The mixture was heated to 50 °C for 2 days in a sealed tube. Then it was sequentially treated with $NaHCO_3$ (2.0 g), water (0.25 mL) and 20 mL of 1:1 CH_2Cl_2-MeOH. After addition of anhydrous $MgSO_4$ the resulting slurry was filtered. The filtrate was concentrated and purified by column chromatography (4-6% MeOH-CH_2Cl_2) to afford 9 mg of compound **3.28** (11 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ

ppm 2.14 (s, 3H), 2.37 (ddd, *J* = 14.90, 7.02, 4.53 Hz, 1H), 2.61 (d, *J* = 14.90 Hz, 1H), 4.02 (dd, *J* = 9.80, 3.46 Hz, 1H), 4.28 (dd, *J* = 9.78, 1.14 Hz, 1H), 4.52 (t, *J* = 3.86 Hz, 1H), 5.94 (d, *J* = 6.56 Hz, 1H), 7.32 (d, *J* = 7.50 Hz, 1H), 8.03 (d, *J* = 7.48 Hz, 1H), 9.39 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 25.12, 41.49, 70.28, 78.87, 89.48, 95.73, 146.47, 155.43, 162.11, 170.95. ESI-HRMS for $[C_{10}H_{13}N_3O_4 + H]^+$ calcd, 240.0979; found, 240.0982 & $[2M + H]^+$ 479.1936.

1′-(Cytosin-1-yl)-2′-deoxy-α-L-threofuranose (3.28):

Method – 1. Applying the reaction conditions described for **3.12** on compound **3.28** (18 mg, 75 μmol) gave 10 mg of compound **3.29** (67% yield) as an amorphous solid.

Method – 2. To a stirring mixture of 1,2,4-triazole (223 mg, 3.24 mmol) in 5 mL of pyridine $POCl_3$ (75 μL, 0.81 mmol) was added dropwise at room temperature. After 10 minutes compound **3.30** (65 mg, 0.27 mmol) was added and stirring was continued for an additional 3h. After that time the reaction was bubbled with ammonia gas for 2h. Since TLC indicated that the deacetylation was not completed, the volatiles were removed and the residue treated with 7N methanolic ammonia (5 mL) for 5h. After evaporation the residue was purified by flash column chromatography (6% MeOH-CH_2Cl_2) to give 19 mg of the title compound **3.29** in 36% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm 1.82 (ddd, *J* = 14.28, 1.40, 1.10 Hz, 1H), 2.40 (ddd, *J* = 14.06, 7.87, 5.59 Hz, 1H), 3.81 (dd, *J* = 9.35, 3.74Hz, 1H), 3.97 (dt, *J* = 9.31, 1.28 Hz, 1H), 4.33 (m, 1H), 5.16 (d, *J* = 2.76 Hz, 1H), 5.69 (d, *J* = 7.42 Hz, 1H), 5.99 (dd, *J* = 7.83, 2.43 Hz, 1H), 7.08 (brd, *J* = 15.32 Hz, 2H), 7.75 (d, *J* = 7.41 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm 41.48, 69.70, 77.26, 86.52, 93.85, 142.46, 155.98, 166.37. ESI-HRMS for $[C_8H_{11}N_3O_3 + H]^+$ calcd, 198.0873; found, 198.0876.

1′-(Uracil-1-yl)-2′-deoxy-3′-*O*-acetyl-α-L-threofuranose (3.30): To a stirring solution of compound **3.26** (55 mg, 0.277 mmol) and 4-dimethyl aminopyridene (DMAP, 50 mg, 0.41 mmol) in 2 mL of DMF acetic anhydride (31 μL, 0.33 mmol) was added. After stirring for 24h, the solvent was evaporated under vacuum and the residue was purified by column chromatography to isolate compound **3.30** (65 mg, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.96 (s, 3H), 2.22 (ddd, *J* = 15.48, 2.74,

1.76 Hz, 1H), 2.58 (ddd, *J* = 15.49, 7.24, 5.81 Hz, 1H), 4.04 (dd, *J* = 10.95, 3.87 Hz, 1H), 4.21 (ddd, *J* = 10.95, 1.60, 0.69 Hz, 1H), 5.31 (app-tt, *J* = 4.80, 0.84 Hz, 1H), 5.68 (d, *J* = 8.18 Hz, 1H), 6.07 (dd, *J* = 7.21, 1.94 Hz, 1H), 7.45 (d, *J* = 8.18 Hz, 1H), 9.20 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 21.24, 39.13, 72.88, 75.39, 86.61, 101.63, 139.77, 150.53, 163.72, 170.19. ESI-HRMS for $[C_{10}H_{12}N_2O_5 + H]^+$ calcd, 241.0819; found, 241.0830.

Phenyl-(benzoxy-L-alaninyl)-phosphorochloridate (3.33): To a stirred solution of phenyldichlorophosphate **3.31** (0.30 mL, 2.00 mmol) and L-alanine benzyl ester tosylate **3.32** (0.43 g, 2.00 mmol) in anhydrous CH_2Cl_2 (15 mL) was added anhydrous TEA (0.56 mL, 4.00 mmol) dropwise under an Ar atmosphere at -78 ^oC. Following the addition the reaction mixture was stirred at -78 ^oC for 30 min, then at room temperature for 2h. Formation of the desired compound was monitored by ^{31}P NMR. After this period the solvent was removed under reduced pressure and the residue triturated with anhydrous diethyl ether. The precipitate was filtered under nitrogen and the solution was concentrated to give yellow oil (87%, 0.62 g). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 7.33-7.28 (10H, m, PhO, OCH_2P*h*), 5.15-5.13 (2H, m, OC*H*$_2$Ph), 4.18-4.13 (1H, m, C*H*NH), 1.46-1.44 (3H, m, CH_3). ^{31}P NMR ($CDCl_3$, 202 MHz) δ ppm 7.86, 7.52.

α-L-Threofuranosyl-1′-(Thymin-1-yl)-2′-deoxy-3′-*O*-[phenyl-(benzoxy-L-alaninyl)]-phosphate (3.34): To a solution of **3.25** (0.093 g, 0.44 mmol) in anhydrous THF (10 mL) was added 1.0M solution of *tert*-butylmagnesium chloride in THF (0.88 mL, 0.88 mmol) and the reaction mixture was stirred under an Ar atmosphere for 30 min. After this period, a solution of **3.33** (0.31 g, 0.88 mmol) in anhydrous THF (5 mL) was added dropwise and the reaction mixture was stirred at room temperature for 18h. Then, 1.0M solution of *tert*-butyl magnesium chloride in THF (0.44 mL, 0.44 mmol) and a solution of 32 (0.15 g, 0.44 mmol) in anhydrous THF (3 mL) was added and the stirring was continued for further 5h. After this period, the solvent was removed and the residue was purified by column chromatography, gradient elution of CH_2Cl_2/MeOH = 98/2 then 96/4 to give a white solid (33%, 0.077 g). ^{1}H NMR (CD_3OD, 500 MHz) δ ppm 7.52 (0.5H, d, J = 1.2 Hz, H-6 of one diastereoisomer),

7.47 (0.5H, d, *J* = 1.2 Hz, H-6 of one diastereoisomer), 7.38-7.30 (7H, m, PhO, OCH_2*Ph*), 7.22-7.16 (2H, m, PhO, OCH_2*Ph*), 7.12-7.10 (1H, m, PhO, OCH_2*Ph*), 6.10 (0.5H, dd, *J* = 7.8 Hz, 2.3 Hz, H-1′), 6.06 (0.5H, dd, *J* = 7.5 Hz, 1.8 Hz, H-1′), 5.21-5.17 (1H, m, H-3′), 5.16, 5.15 (2H, 2s, OC*H*$_2$Ph), 4.45 (0.5H, dd, *J* = 10.8 Hz, 1.7 Hz, H-4′ of one diastereoisomer), 4.37 (0.5H, dd, *J* = 10.8 Hz, 1.7 Hz, H-4′ of one diastereoisomer), 4.03-3.92 (2H, m, H-4′, C*H*CH_3), 2.63-2.55 (1H, m, H-2′), 2.32 (0.5H, d, *J* = 15.5 Hz, H-2′ of one diastereoisomer), 2.24 (0.5H, d, *J* = 15.5 Hz, H-2′ of one diastereoisomer), 1.83 (1.5H, d, *J* = 1.1 Hz, 5-CH_3 of one diastereoisomer), 1.82 (1.5H, d, *J* = 1.1 Hz, 5-CH_3 of one diastereoisomer), 1.36 (1.5H, d, J = 0.9 Hz, CHC*H*$_3$ of one diastereoisomer), 1.34 (1.5H, d, *J* = 1.0 Hz, CHC*H*$_3$ of one diastereoisomer). ^{13}C NMR (CD_3OD, 125 MHz) δ ppm 12.71 (5-CH_3), 20.16 (d, J_{C-P} = 7.5 Hz, CH*C*H_3), 20.33 (d, J_{C-P} = 6.5 Hz, CH*C*H_3), 40.51 (d, J_{C-P} = 5.6 Hz, C-2′), 40.73 (d, J_{C-P} = 3.9 Hz, C-2′), 51.60, 51.84 (2s, *C*HCH_3), 68.00, 68.04 (2s, O*C*H_2Ph), 76.34 (d, J_{C-P} = 4.0 Hz, C-4′), 76.79 (d, J_{C-P} = 5.5 Hz, C-4′), 77.95 (d, J_{C-P} = 5.2 Hz, C-3′), 77.99 (d, J_{C-P} = 4.9 Hz, C-3′), 86.95, 87.47 (2s, C-1′), 110.70, 111.03 (2s, C-5), 121.09, 121.14, 121.18, 126.26, 126.29, 129.41, 129.43, 129.64, 129.65, 130.82, 130.92 (PhO, OCH_2*Ph*), 137.29 (‘ipso’ OCH_2*Ph*), 137.77, 137.80 (2s, C-6), 152.03, 152.07, 152.12, 152.22, 152.31 (C-2, (‘ipso’ O*Ph*), 166.49, 166.55 (2s, C-4), 174.49 (d, J_{C-P} = 4.6, *C*OOCH_2Ph), 174.91 (d, J_{C-P} = 4.1, *C*OOCH_2Ph). ^{31}P NMR (CD_3OD, 202 MHz) δ ppm 2.92, 2.27. ES-MS= 528.16 (M run on negative mode). HPLC = H_2O/AcCN from 95/5 to 0/100 in 30 min = retention time 13.87, 14.19 min.

3.4.2. Pharmacological assay procedures

3.4.2.1. Carboxypeptidase Y enzymatic assay

Compound **3.34** (5.5 mg) was dissolved in d_6-acetone (150 μL), and Trizma buffer (pH 7.6) (300 μL) was added. The resulting cloudy solution was placed in a NMR tube and a ^{31}P NMR experiment at 25 °C was recorded as the blank experiment. Then

a solution of carboxypeptidase Y (0.1 mg) in Trizma buffer (150 μL) was added and a ^{31}P NMR experiment was performed recording the experiment every 5 min.

3.4.2.2. Cytostatic activity assay

The compounds were evaluated for their potential cytostatic activities against murine leukemia L1210, human CD_4^+ T-cell lymphocyte CEM and human cervix carcinoma HeLa cancer cells. In brief, different concentrations (5-fold dilutions) of the compounds were incubated at 37°C for 72h (HeLa and CEM cells) or 48h in the L1210 bioassay. After the incubation period, the number of viable cells were counted by a Coulter Particle counter and the IC_{50} were defined as the 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

3.4.2.3. Nucleoside kinase assay

The activity of recombinant thymidine kinase (TK), TK-2, herpes simplex virus-1 (HSV-1) TK and varicella-zoster virus (VZV) TK, and the 50% inhibitory concentration of the test compounds were assayed in a 50 μL reaction mixture containing 50 mM Tris/HCl, pH 8.0, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM CHAPS, 3 mg/mL bovine serum albumin, 2.5 mM ATP, 1 μM [methyl-^{3}H]dThd, and enzyme. The samples were incubated at 37 °C for 30 min in the presence or absence of different concentrations (5-fold dilutions) of the test compounds. At this time point, the enzyme reaction still proceeded linearly. Aliquots of 45 μL of the reaction mixtures were spotted on Whatman DE-81 filter paper disks (Whatman, Clifton, NJ). The filters were washed three times for 5 min each in 1 mM ammonium formate, once for 1 min in water, and once for 5 min in ethanol. The radioactivity was determined by scintillation counting.

3.4.2.4. Antiviral assays

The anti-HIV activity was evaluated against the laboratory HIV-1 strain IIIB and HIV-2 strain ROD in human T-lymphocyte CEM cell cultures. Briefly, virus stocks were titrated in human T-lymphocyte CEM cells and expressed as the 50% cell culture infective dose ($CCID_{50}$, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures). CEM cells were suspended in culture medium at ~ 3 x 10^5 cells/ml and infected with HIV at ~ 100 $CCID_{50}$. Immediately after viral exposure, 100 µl of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4-day incubation period at 37 °C, the giant cell formation was microscopically determined. Compounds were tested in parallel for their potential cytostatic effects in uninfected CEM cell cultures.

The other antiviral assays for herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), VZV (YS) and CMV (Davis and AD-169) were based on inhibition of virus-induced cytopathicity in HEL cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus or 20 plaque forming units (VZV) in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds

3.4.2.5. Spectrophotometric binding assay for TMPK*mt*

TMPK*mt* activities were determined using the coupled spectrophotometric assay described by Blondin *et al.*[167] using an Eppendorf ECOM 6122 photometer and a wavelength of 334 nm. The reaction medium (0.5 mL final volume) contained 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each of lactate dehydrogenase, pyruvate kinase and nucleoside diphosphate kinase. The concentrations of ATP and dTMP were kept constant at 0.5 and 0.05 mM, respectively, whereas the concentrations of analogues varied between 0.01 and 2 mM.

CHAPTER – 4

TURNING APIONUCLEOS(T)IDES INTO ANTIVIRALS

4.1. Objectives

2′,3′-β-D-Dideoxyapio-D-furanose nucleosides (ddANs, **4.1**, Figure 4.1) were synthesized in the early 90s for their potential antiviral properties, but were found inactive. However, it was recently shown that the 3′-*O*-phosphonomethylated A and T analogues **4.4** exhibit promising anti-HIV properties. Since these phosphonates act as bioisosteres of the phosphorylated species **4.5**, we decided to reinvestigate the biological activity of these ddANs and expand their potential as antiviral agents by synthesizing the corresponding phosphoramidate prodrugs **4.6**,[168] which would ideally lead to the intracellular release of the parent nucleotide **4.5**, thereby by-passing the often problematic first phosphorylation step in the conversion to the active triphosphate species. We envisioned a synthetic approach that would also give access to the known apionucleosides **4.3** and their 3′-deoxy counterparts **4.2**.

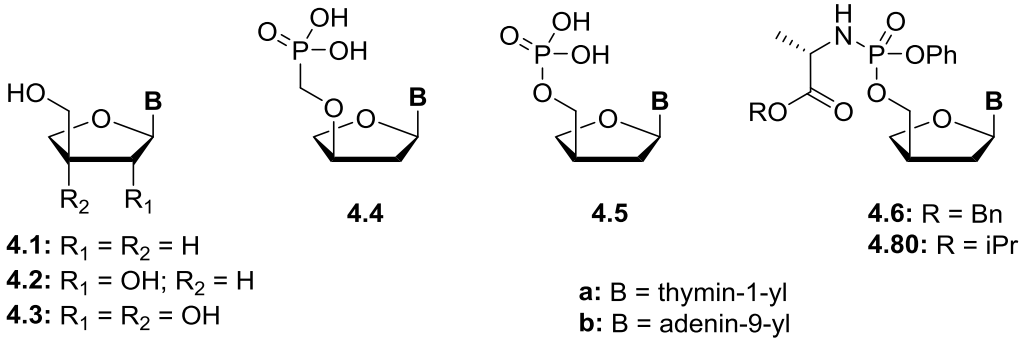
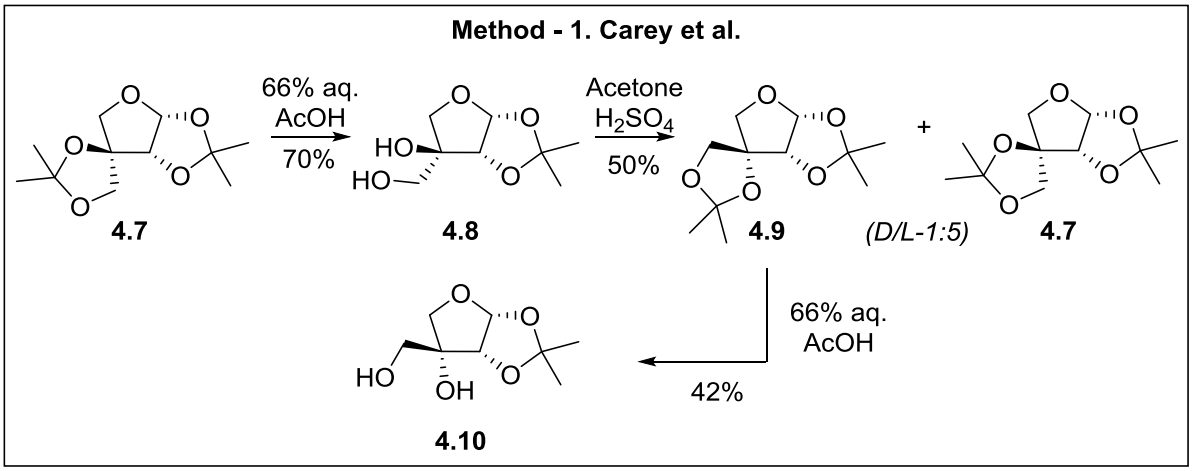


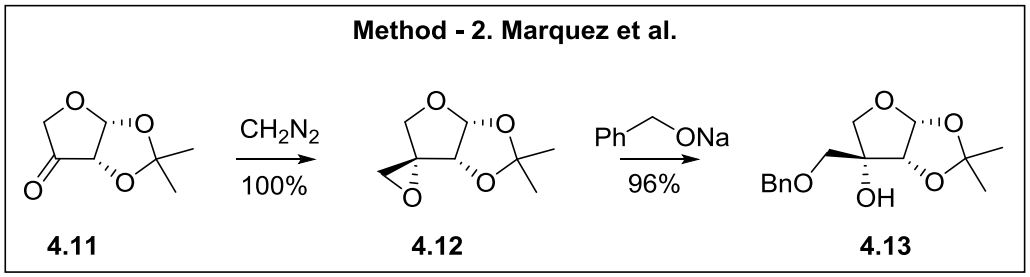
Figure 4.1. Apionucleosides and their Phosphates

4.2. Reported Methods to Synthesize Useful Apiofuranose Intermediates

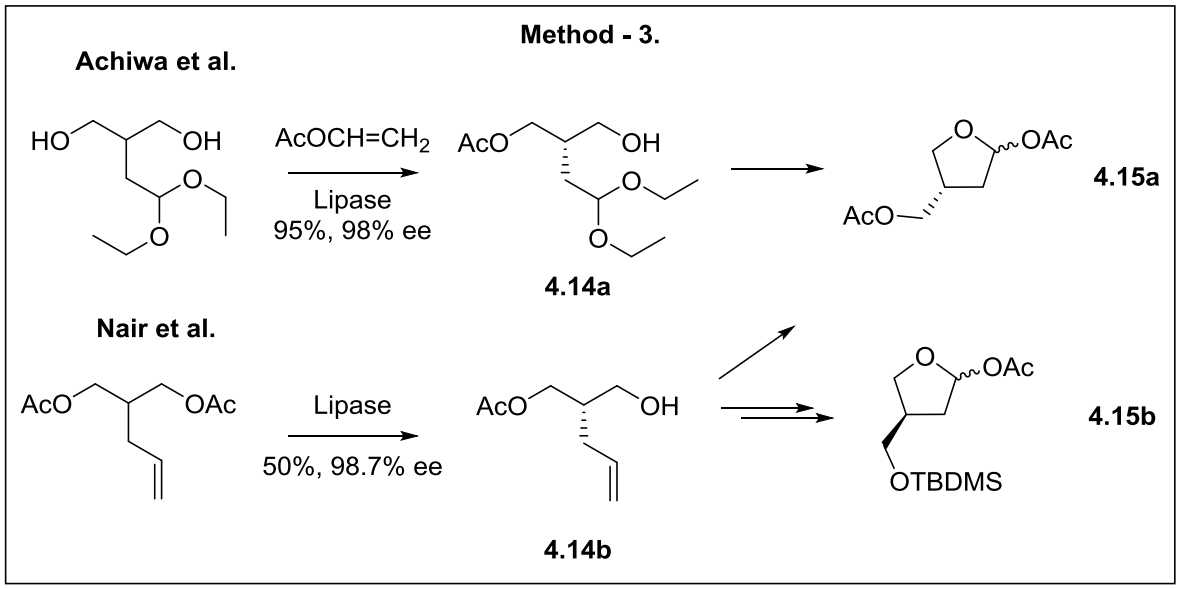
Several methods are known for the preparation of apiofuranose intermediates or their 3-deoxy analogues, suitable for the construction of the corresponding nucleosides. Carey *et al.* described the regioselective hydrolysis of the 3,5-*O*-isopropylidene moiety of 1,2:3,5-di-*O*-isopropylidene-α-D-apio-D-furanose (**4.9**) to access **4.10** in a single step (Method-1). [169] Compound **4.9** is synthesized from the expensive commercially available material **4.7**. An additional drawback of this method is that, the sulfuric acid catalyzed equilibration step gives the required product in low yield.



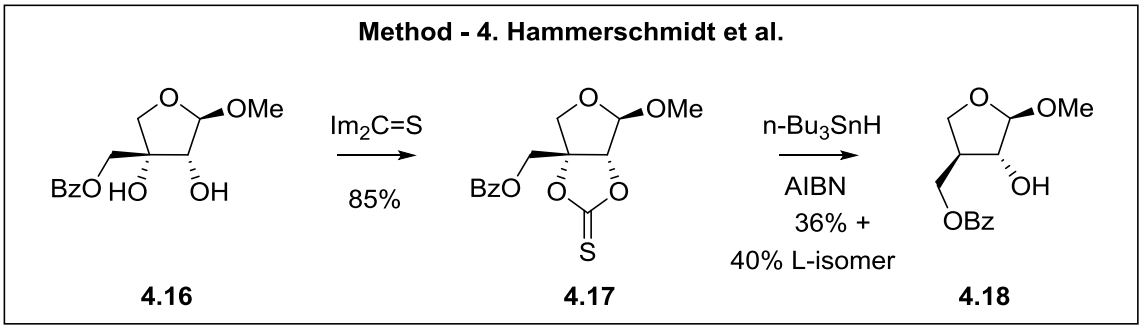
Marquez and coworkers employed the explosive reagent diazomethane to convert ketone **4.11** to epoxide intermediate **4.12**, which was then treated with sodium benzylalkoxide to open the spiro-oxirane to afford intermediate **4.13** (Method-2).[170]



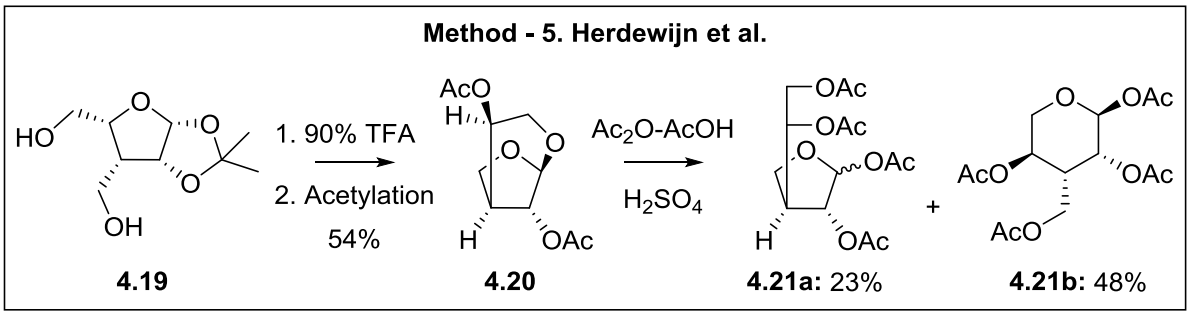
The very first synthesis of D-dideoxyapio-L-furanose nucleoside was realized by lipase catalyzed asymmetrization (Method-3).[16] The method was modified and applied to the synthesis of both D-dideoxyapio-D- and L-furano nucleosides by Nair and coworkers via intermediate **4.14b**.



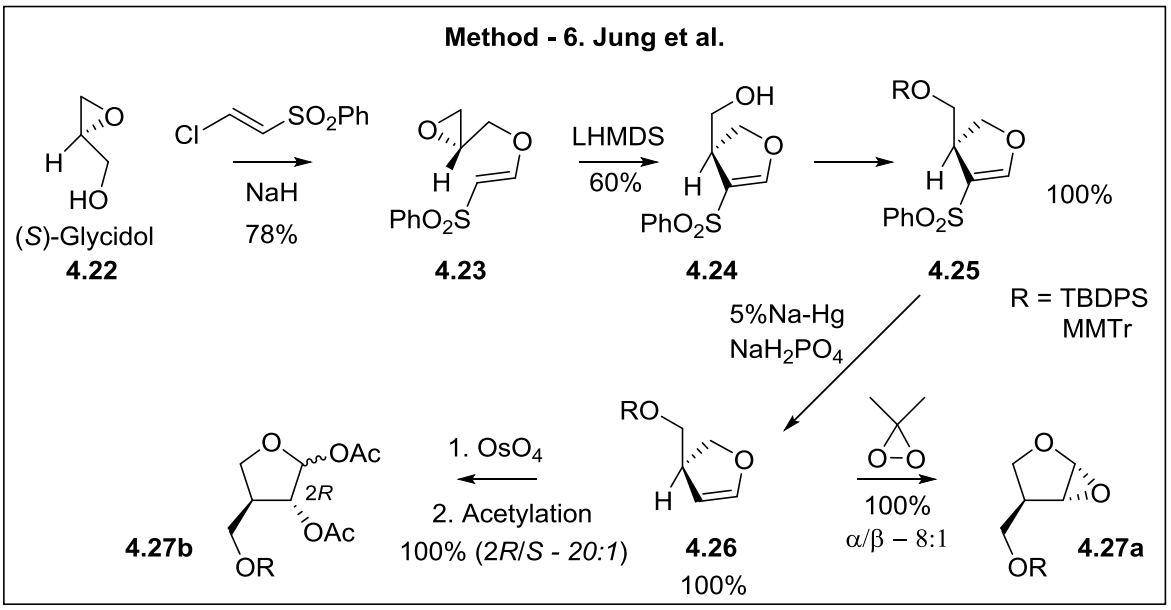
A Barton-McCombie deoxygenation was used by Hammerschmidt *et al.* to synthesize 3-deoxyapio sugar **4.18** (Method-4).[24] Though straightforward, this method provides a mixture of D- and L-furanosides, thus lacking selectivity.



Herdewijn *et al.* reported the formation of 3-deoxy-3-C-branched furanose **4.21a** as a minor product while synthesizing pyranose nucleosides.[171] The compound results from opening of bicyclic acetal **4.20**. Compound **4.19** was accessed in five steps from suitably protected L-arabinofuranose.

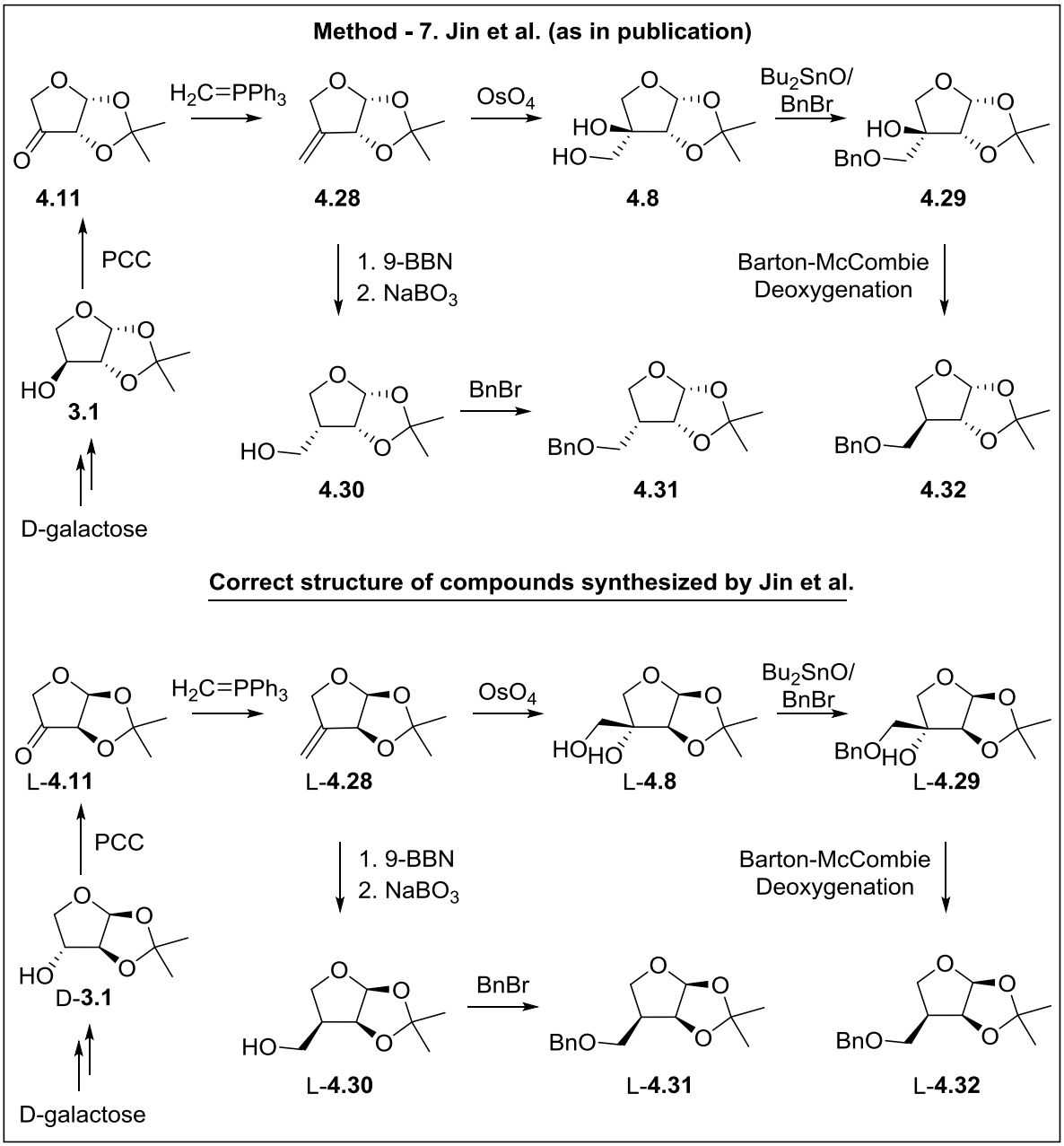


Unlike the previously mentioned chiral pool strategies, Jung *et al.* used a *de novo* approach starting from allyl alcohol that was converted to (*S*)-glycidol (**4.22**), via Sharpless asymmetric epoxidation. The latter was converted to the 3-deoxy-apio-furanose coupling partners **4.27a** or **4.27b** in good yields (Method-6).[27] It should be noted that the method is not suitable for the simultaneous synthesis of the non-deoxygenated apionucleosides in which we are also interested.



Jin and coworkers reported a multistep protocol involving the volatile intermediate **4.28** (Method-7).[26] In our hands the Wittig reaction on ketone **4.11** turned out to be low yielding. The exomethylene compound **4.28** was stereoselectively dihydroxylated using osmium tetroxide, followed by dibutyltin oxide mediated selective benzylation. The authors claim that Barton-McCombie deoxygenation gave the 3-deoxy-D-apio-D-furanose intermediate **4.32**. Subsequent reactions with this deoxygenated product failed to produce the desired nucleosides. We discovered that Jin *et al*. used D-galactose as starting material. Hence, they actually synthesized the enantiomer of **3.1**, *i.e.* 1,2-*O*-isopropylidene-α-D-threofuranose (D-**3.1**). Based on careful analysis of the reported ^{1}H NMR data and their comparison with those of the two possible 3′-epimers, we are confident that the stereochemistry of the deoxygenated product obtained by Jin *et al.* was wrongly assigned and actually corresponds to the L-apio isomer L-**4.31** (please refer to Scheme 4.1 & 4.7 for **4.31** and **4.32** respectively).

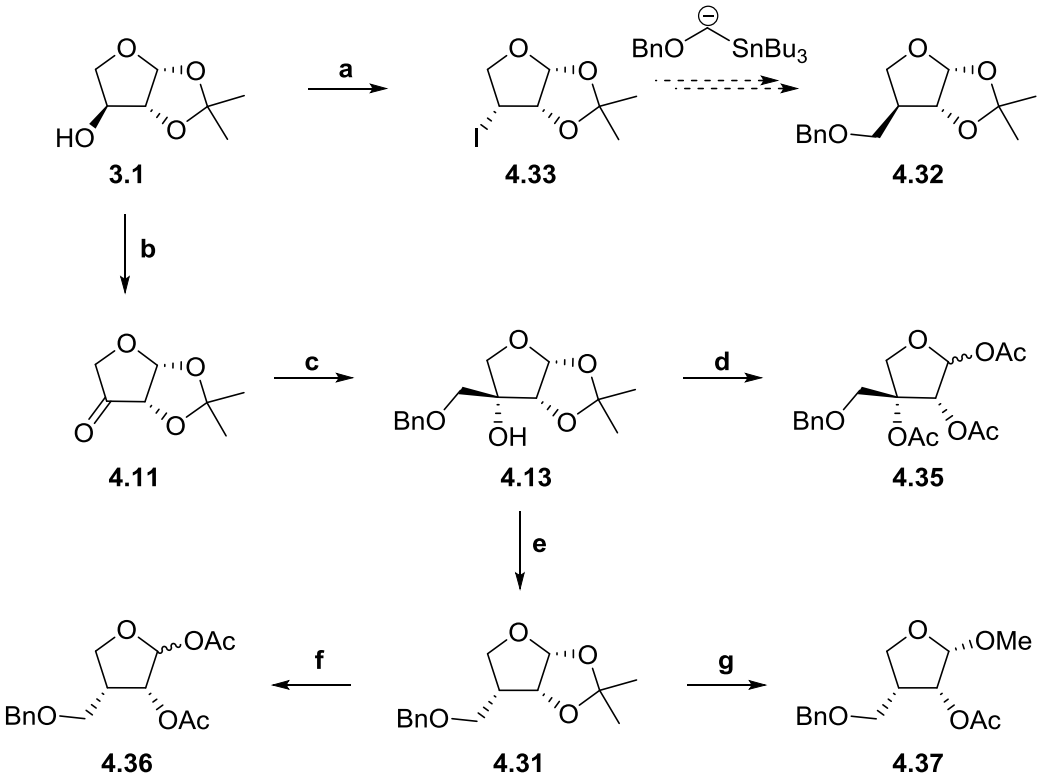
Because all previous methods suffer from limitations, we present an optimized procedure to arrive at the desired nucleosides.



4.3. Results and Discussion

4.3.1. Chemistry

4.3.1.1. Syntheses of α-D-apio-L-furanonucleosides



Scheme 4.1. Synthesis of the D-apio D- and L-furanose coupling partners **4.35**, **4.36** and **4.37**. *Reagents and conditions:* (a) I_2, PPh_3, imidazole, toluene, 115 °C, 3h, 22%; (b) TEMPO, BAIB, CH_2Cl_2, rt, 3-4h, 90%; (c) $BOMSnBu_3$, *n*-BuLi, THF, -78 °C, 2h, 68%; (d) (i) 80% aq. AcOH, 80 °C, 8h; (ii) Ac_2O, DMAP, pyridine, 55 °C, 16h, 75%; (e) (i) NaH, CS_2, MeI, THF, 0 °C → rt, 1h; (ii) Et_3B, Bu_3SnH, toluene, rt, 3-4h, 68%; (f) (i) 80% aq. AcOH, 80 °C, 8h; (ii) Ac_2O, DMAP, rt, pyridine, 4h, 57%; (g) (i) *p*-TSA, MeOH, rt, overnight; (ii) Ac_2O, DMAP, pyridine, 0 °C → rt, 4h, 77%.

1,2-*O*-isopropylidene-α-L-threose (**3.1**, Chapter 3), used as a starting material in this new route, was synthesized following a reported procedure. Initially, a synthetic route involving the iodo-compound **4.33** was planned, but the low yield in the first step rendered this protocol unattractive. Interestingly, screening of different oxidation methods to convert **3.1** to ketone **4.11** indicated that TEMPO-BAIB oxidation, which

is best known for oxidation of primary hydroxyl groups, was the most effective. Several variations of the polarity reversal concept were explored to introduce the desired carbon homologation. The reaction of benzyloxymethyl chloride in the presence of samarium iodide did not yield the desired product (Table 4.1), while the corresponding Grignard reaction gave **4.13** in disappointing yields (25%). [172] Nucleophilic attack of the ketone with lithiated benzyloxymethyltributyltin at -78 °C afforded **4.13** in a moderate yield (68%),[173] which may be ascribed to the propensity of compound **4.11** to undergo self-condensation to the aldol dimer.[174]

Table 4.1. Conditions for the conversion of compound **4.11** to **4.13**

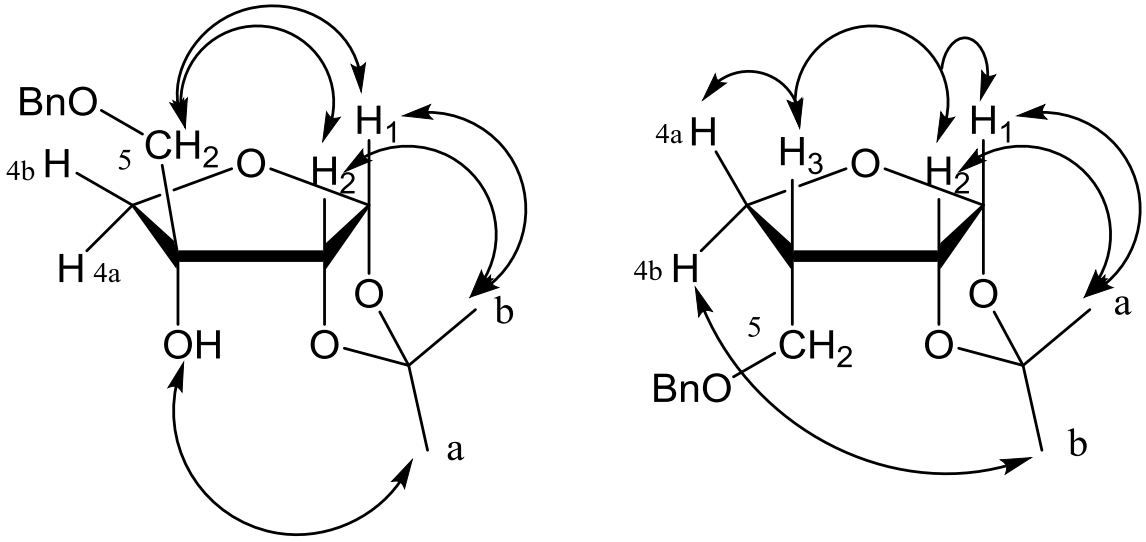
Entry	*Reagent*	*Additive*	*Solvent/temp*	*Yield*
1	*BOMCl*	*SmI_2 (2.2 eq)*	*THF/0 °C*	*0%*
2	*BOM-MgCl*	*$HgCl_2$ (0.2 eq)*	*THF/-78 °C*	*25%*
3	*BOM-Sn(nBu)$_3$, BuLi*	*none*	*THF/-78 °C*	*68%*

The stereochemistry of **4.13** was confirmed by the fact that its NMR spectra were in accordance with reported data[170] as well as by a 2D ^{1}H-^{1}H NOESY experiment (Figure 4.2 and 4.3). The nOe interactions between the anomeric proton (δ_{ppm} 5.76 ppm) and 3-CH_2 protons (δ_{ppm} 3.46, 3.56), between 2-H (δ_{ppm} 4.39) and 3-CH_2, between 3-OH (δ_{ppm} 2.85) and 4-Hα (δ_{ppm} 3.71) and iPr-CH_3 (δ_{ppm} 1.58) are in agreement with the stereochemical assignment of **4.13**. One-pot acid hydrolysis and acetylation of **4.13** gave the tri-acetylated apiose **4.35** in 75% yield in a 2:1 α/β anomeric ratio.

Barton-McCombie deoxygenation of **4.13** afforded the 3-deoxy *threo* isomer **4.31,** which is explained by radical quenching from the least hindered face, *i.e.* opposite to the isopropylidene comprising face.[175] The stereochemistry of **4.31** was deduced by the absence of nOe for anomeric proton (δ_{ppm} 5.83 ppm, Figure 4.2 and 4.4) with 3-CH_2 (δ_{ppm} 3.69, 3.78) and the presence of relatively intense nOe cross-peaks between 3-Hα (δ_{ppm} 2.37-2.52) and 4-Hα (δ_{ppm} 4.01) as compared to 4-Hβ (δ_{ppm} 3.69), in

addition to intense nOe for 2-H (δ_{ppm} 4.65) with anomeric proton and 3-H in 2D ^{1}H-^{1}H NOESY experiment. The structure was further confirmed by the synthesis of C3-epimer (Scheme 4.7). The NMR data of **4.31** match with those reported by Jin *et al.*, who incorrectly assigned those to the 3-deoxy *erythro* intermediate **4.32**.

Compound **4.31** was then hydrolyzed and acetylated to give **4.36** in a 4:1 anomeric ratio. The anomeric configuration was inferred from the anomeric proton coupling constants, *i.e.* 0 Hz for the β-isomer and 4.4 Hz for the α-isomer. However, this conversion lacked reproducibility, especially on a larger scale. To overcome this problem, methyl glycoside **4.37** was synthesized in two steps from **4.31** in 77% yield. β-assignment of the methoxy group in **4.37** is based on the coupling constant of the anomeric hydrogen (0 Hz).



Compound **4.13** Compound **4.31**

Figure 4.2. Observed nOe interactions for compound **4.13** and **4.31**

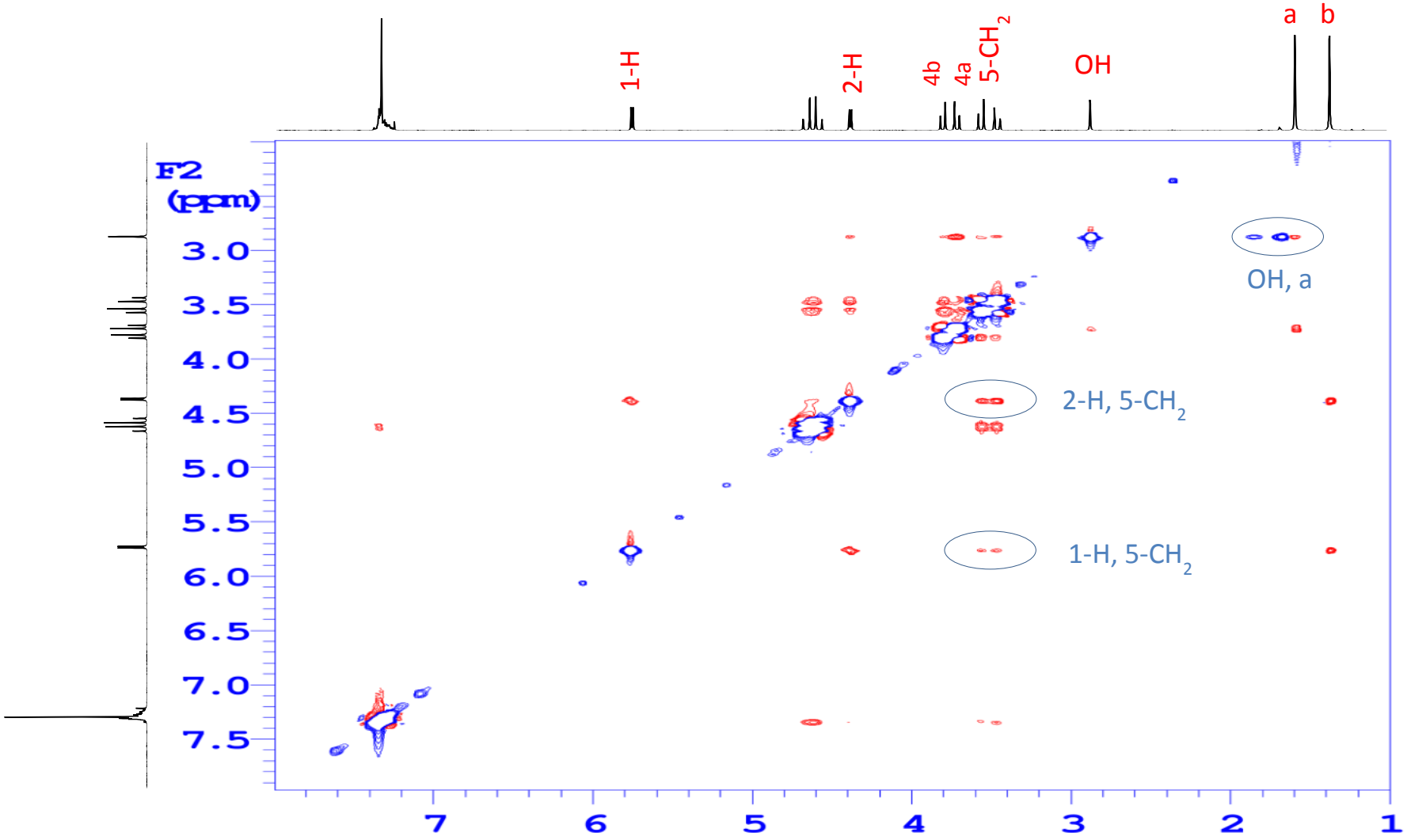


Figure 4.3. 2D ^{1}H-^{1}H NOESY spectrum of compound **4.13**

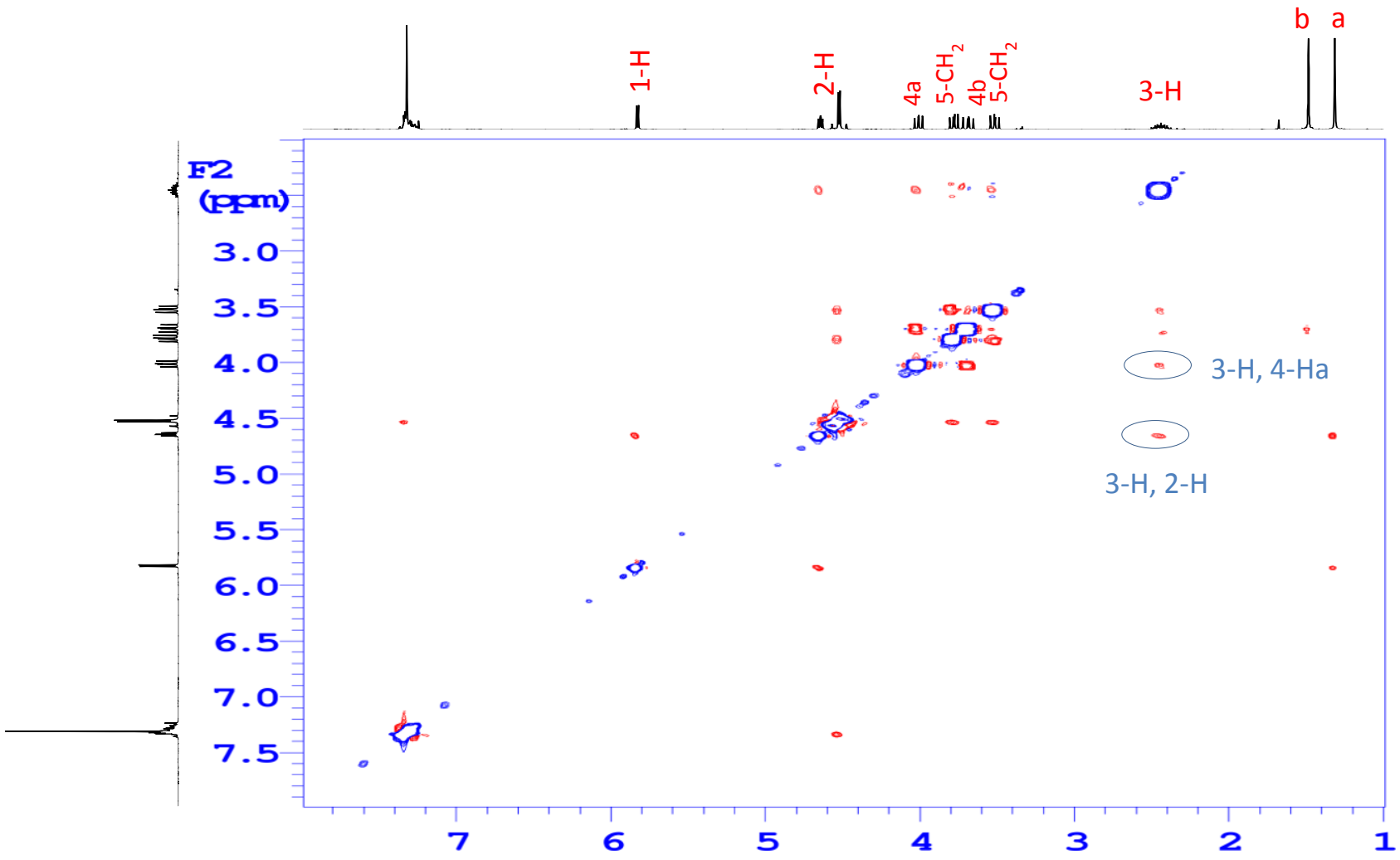


Figure 4.4. 2D ^{1}H-^{1}H NOESY spectrum of compound **4.31**

The diisopropylidene derivative of D-apio-D-furanose was purchased from Carbosynth (Compton, Berkshire, UK, Figure 4.5). However, in the course of our work we discovered that the chemical supplied was the diisopropylidene derivative of D-apio-L-furanose **4.7** (Scheme 4.2).

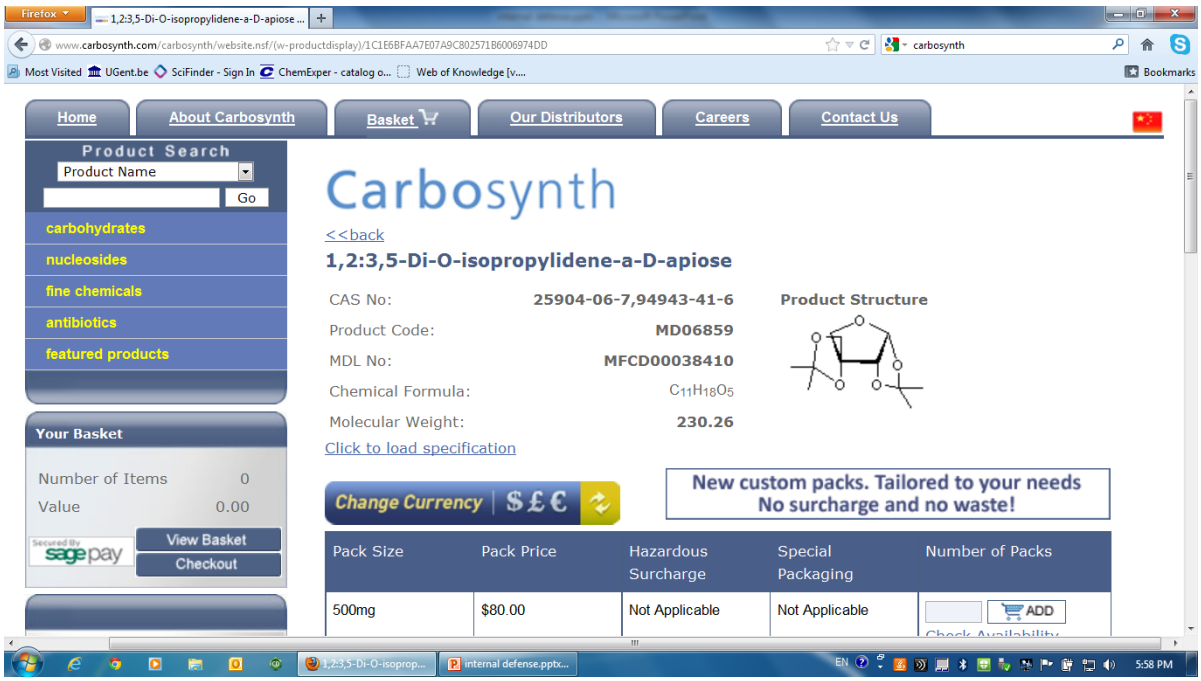
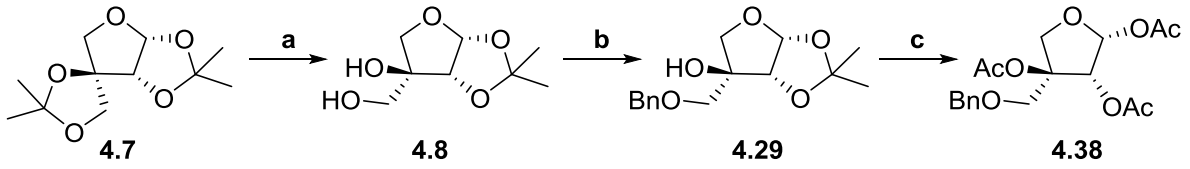
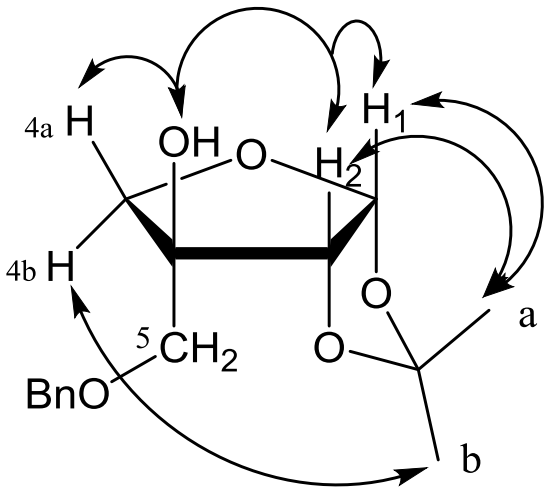


Figure 4.5. A snapshot of the Carbosynth website



Scheme 4.2. Synthesis of D-apio-L-furanose coupling partner **4.38**. *Reagents and conditions:* (a) CH_3COOH-H_2O (2:1), rt, 3 days, 83%; (b) Bu_2SnO, toluene, 140 °C, 2h, TBAB, BnBr, 100 °C, 18h, 94%; (c) (i) 80% aq. AcOH, 80 °C, 8h; (ii) Ac_2O, DMAP, pyridine, 55 °C, 16h, 75%.

The 3,5-*O*-isopropylidene was removed selectively according to the procedure by Carey *et al.* and *O*-benzyl protection was performed as reported by Marquez *et al.* The ^{1}H NMR spectrum of **4.29** proved identical to that obtained by Jin *et al.* (Section 4.3, method-7). In addition, the relative stereochemistry was confirmed by a 2D ^{1}H-^{1}H NOESY experiment (Figure 4.6 and 4.7). As expected and in contrast to **4.13**, the 3-CH_2 protons (δ_{ppm} 3.54, 3.80) failed to show a nOe interaction with the anomeric (δ_{ppm} 5.98) and C-2 (δ_{ppm} 4.35) protons; on the other hand the 3-OH (δ_{ppm} 2.76) showed interactions with 2-H, 4-H, 3-CH_2 but not with the isopropylidene protons (δ_{ppm} 1.48). These nOe peaks fit with an orientation of the 3-CH_2 that is opposite to that of the anomeric and C-2 protons, *i.e*. compound **4.29.** Hydrolysis and acetylation of **4.29** gave **4.38** in good overall yield.



Compound **4.29**

Figure 4.6. Observed nOe interactions for compound **4.29**

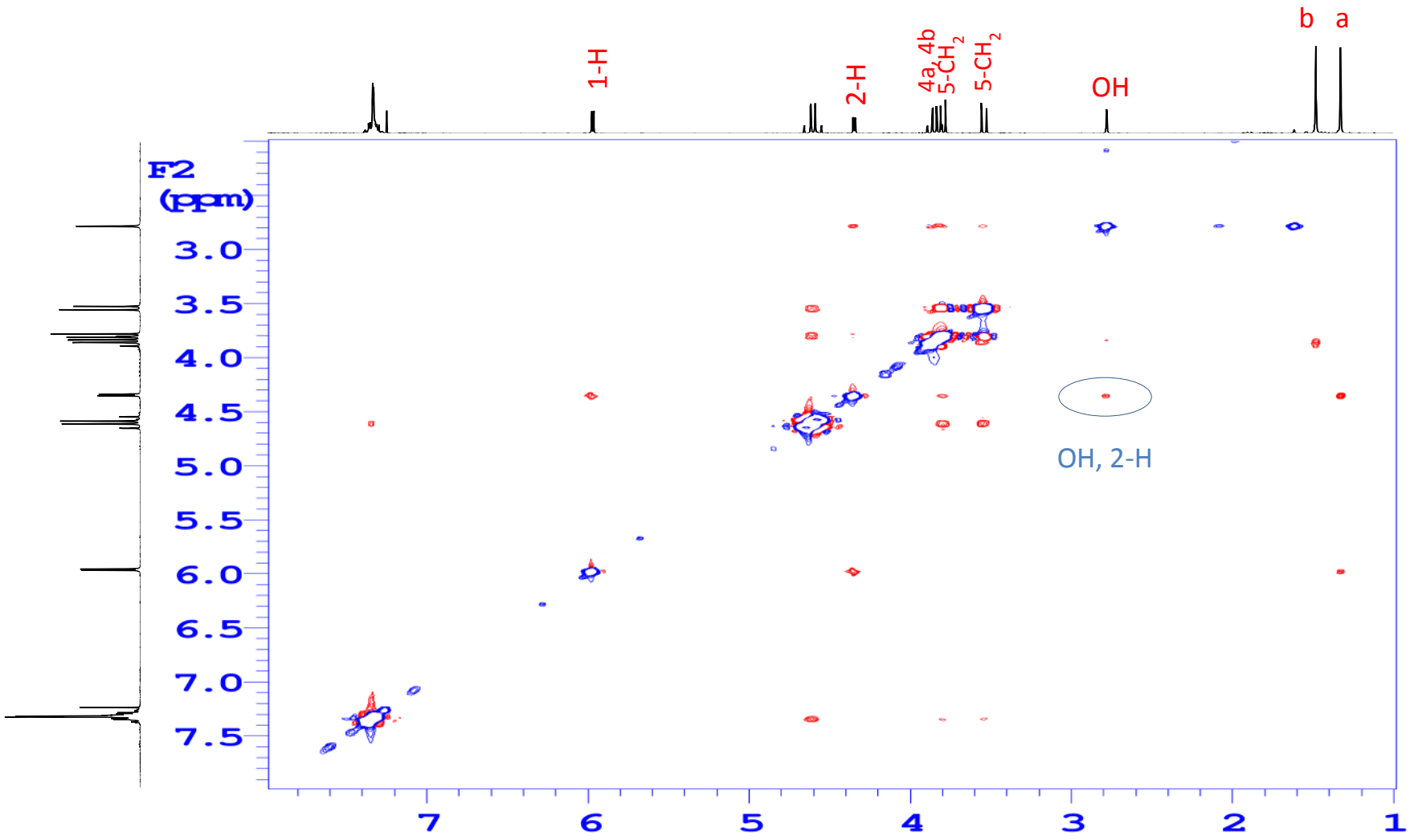
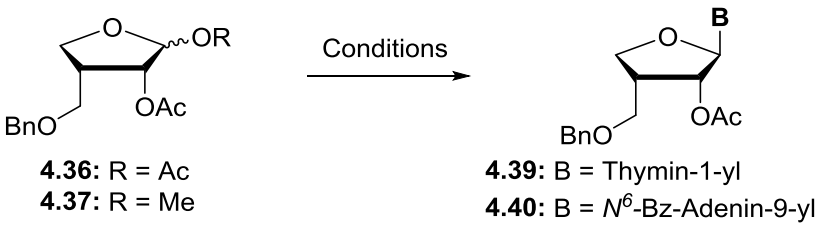


Figure 4.7. 2D ^{1}H-^{1}H NOESY spectrum of compound **4.29**

Having **4.35**, **4.36**, **4.37** and **4.38** in hand, we set out different coupling reactions between **4.36** or **4.37** and silylated thymine or N^6-benzoyladenine under Vorbrüggen conditions (Table 4.2).



4.36: R = Ac
4.37: R = Me

4.39: B = Thymin-1-yl
4.40: B = N^6-Bz-Adenin-9-yl

Table 4.2. Vorbrüggen coupling conditions.

Entry	Sugar	Silylated nucleobase	Reagent	Conditions	Product (yield)[a]
1	**4.36**	T	TMSOTf	1,2-$(CH_2)_2Cl_2$, rt, 4h	**4.39** (quant)
2	**4.36**	N^6-BzA	TMSOTf	1,2-$(CH_2)_2Cl_2$, 40 °C, 48h	**4.40** (32%)[b]
3	**4.37**	T	TMSOTf	1,2-$(CH_2)_2Cl_2$ or CH_3CN, rt, 4h	**4.42**[c]
4	**4.37**	T	$SnCl_4$	CH_3CN, rt, 4h	**4.42**[c]
5	**4.37**	N^6-BzA	$SnCl_4$	CH_3CN, rt, 4h	-
6	**4.37**	T	TMSOTf[d]	CH_3CN, 150 °C, 5 min. microwave	**4.39** [e] + β-anomer (78%)
7	**4.37**	N^6-BzA	TMSOTf[d]	CH_3CN, 150 °C, 5 min. microwave	**4.40** (60 %)

[a] isolated yields, "-" indicates an unresolvable reaction mixture.
[b] the 2′-acetyl analogue of **4.41** was isolated in equal amount.
[c] two diastereomers observed by TLC and HRMS analysis.
[d] 0.2 equivalents of TMSOTf.
[e] inseparable 1:2 mixture of **4.43** and its α-isomer.

Whereas the acetate anomer **4.36** reacted smoothly at room temperature in 4h with silylated thymine in the presence of TMSOTf to give **4.39** in quantitative yield, its coupling with N^6-benzoyladenine proved to be more challenging. The desired coupling product **4.40** was obtained in 32% yield by heating the reaction mixture at 40 °C for 48h. This low yield resulted from the formation of an equal amount of an unknown isomer. ^{1}H NMR of this isomer suffered from peak broadening and indicated the presence of minor impurities. Its UV spectrum (λ_{max} = 331.9 nm) and ^{13}C chemical shifts are characteristic of an N^1-isomer.[158] After treatment with methanolic ammonia for two days, a product was formed that was confidently identified as **4.41** (Figure 4.8). The binding topology of the adenine base to the sugar was determined

with NMR as follows. A correlation between H-1′ (δ_{ppm} 6.61) and C-2 (δ_{ppm} 141.83/ A7) in a 2D ^{1}H-^{13}C HMBC spectrum indicates that the adenine is either bound to N-1 or N-3 (Figure 4.9). In the 2D ^{1}H-^{1}H NOESY spectrum (Figure 4.8 and 4.10), strong nOe cross-peaks were detected between the *ortho* protons of benzoyl (δ_{ppm} 8.22) and protons of the sugar moiety, notably H-1′, H-2′ (δ_{ppm} 4.68) and 2′-OH (δ_{ppm} 4.25). These nOe's are improbable if the base would be attached to N-3, since in this case the benzoyl group and the sugar moiety would be positioned para relative to one another and be spatially too far apart.

Coupling reaction between methylglycoside **4.37** and silylated thymine (Table 4.2; entries 3 and 4), using either TMSOTf or $SnCl_4$, resulted in the formation of two main products that gave spots with comparable intensity on TLC. ESI-HRMS analysis allowed identifying these products as the two diastereomers of **4.42**. The condensation reaction of methylglycoside **4.37** with silylated benzoyladenine in the presence of anhydrous $SnCl_4$ gave an unresolvable reaction mixture.

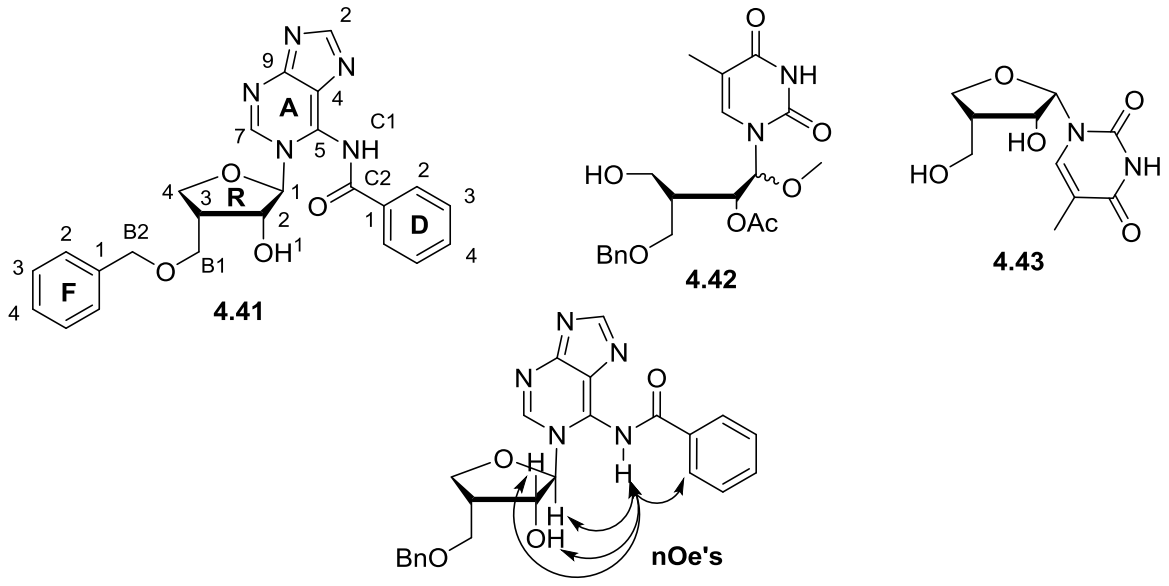


Figure 4.8. Byproducts or deprotected forms of products formed during Vorbrüggen coupling and observed nOe interactions confirming structure **4.41**.

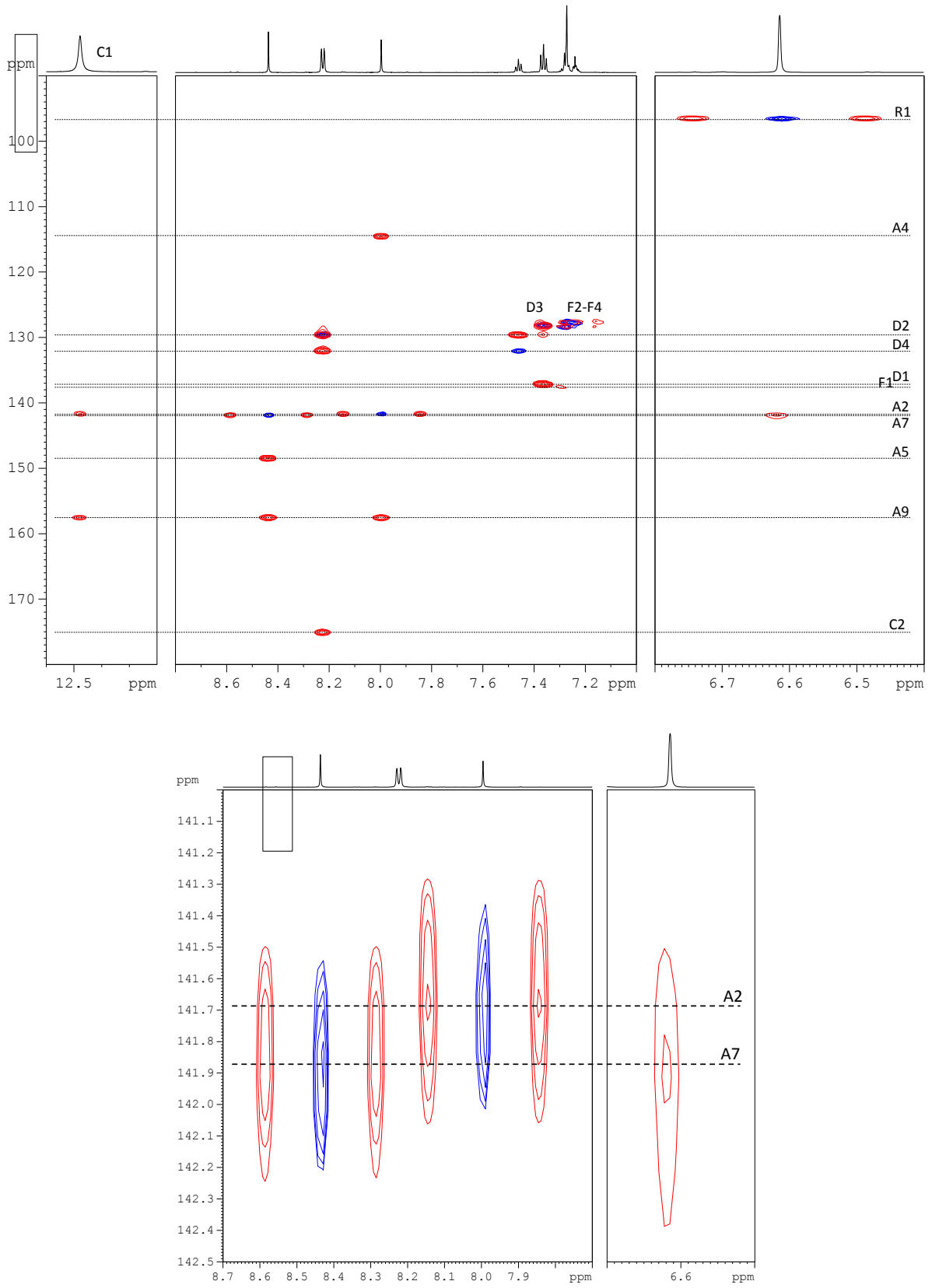


Figure 4.9. Overlay of the HSQC (blue) and HMBC 8 Hz (red) spectra of **4.41** (top) and zoom on the A2 and A7 cross-peaks (bottom).

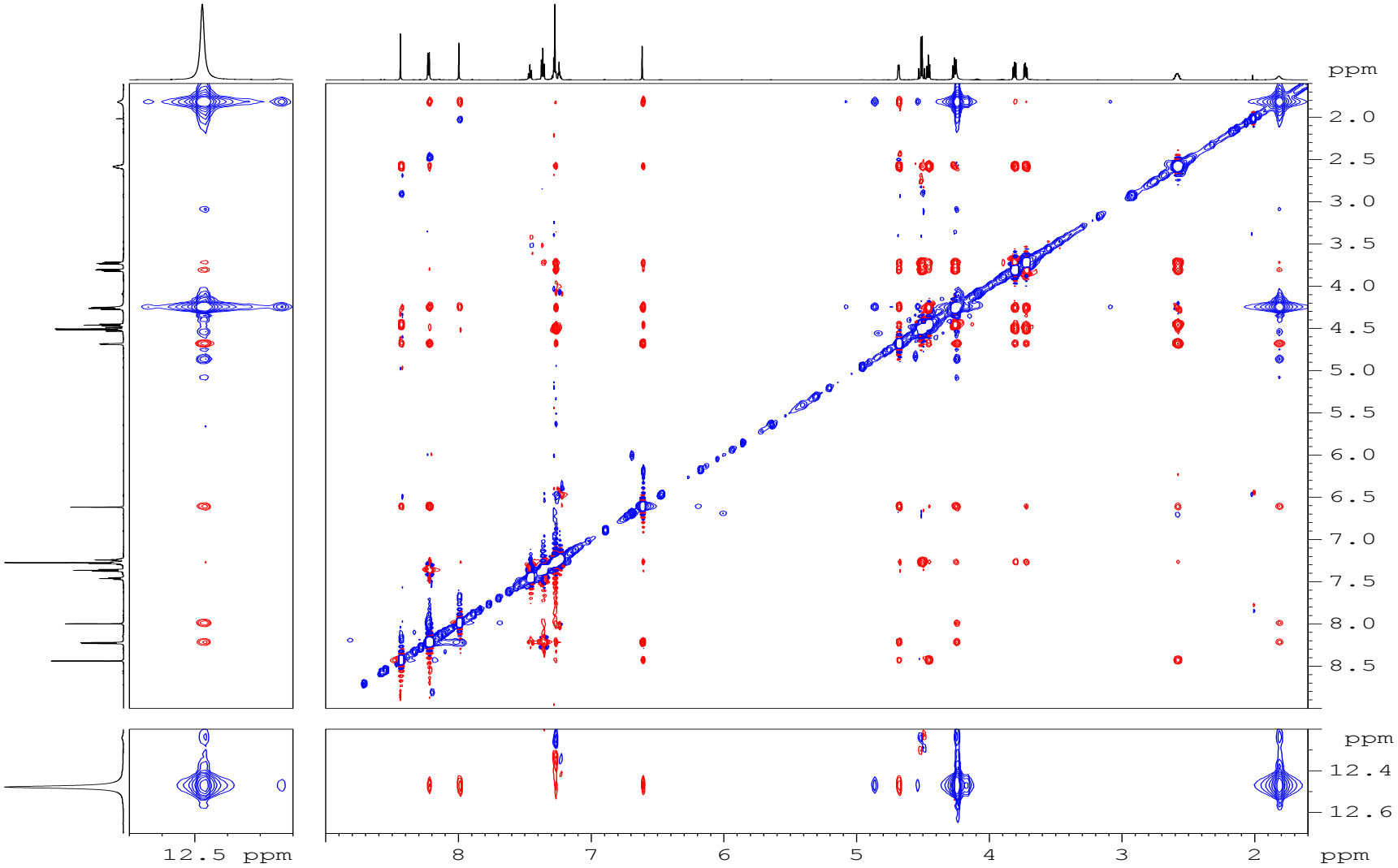
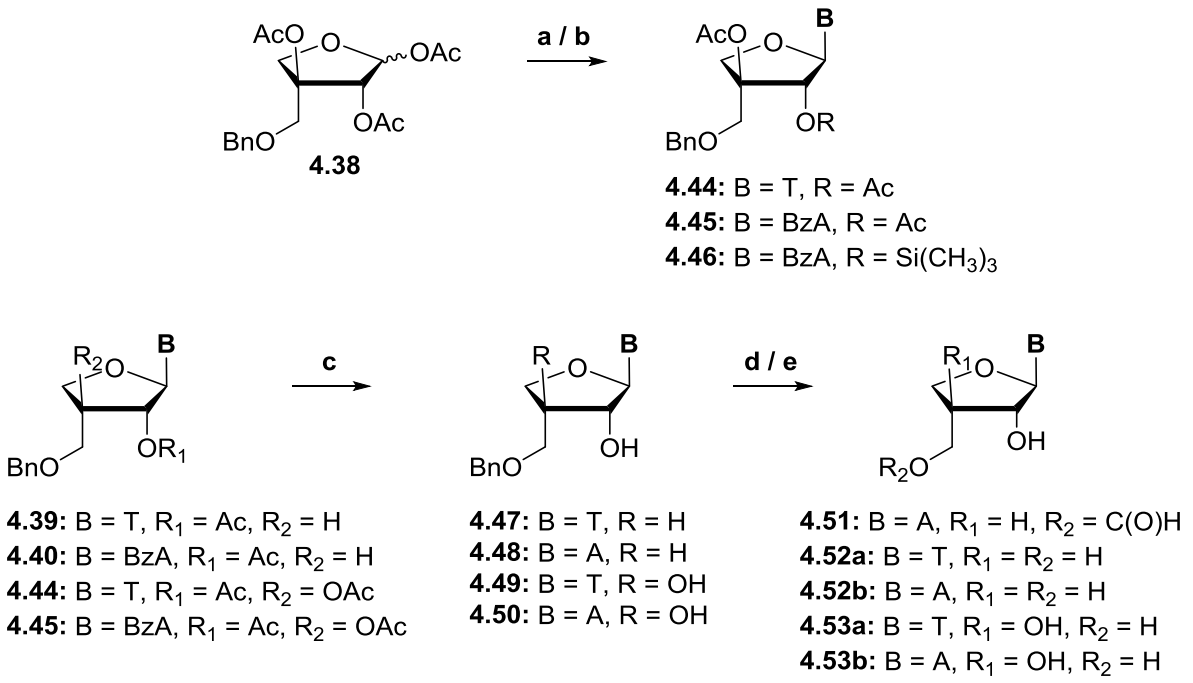


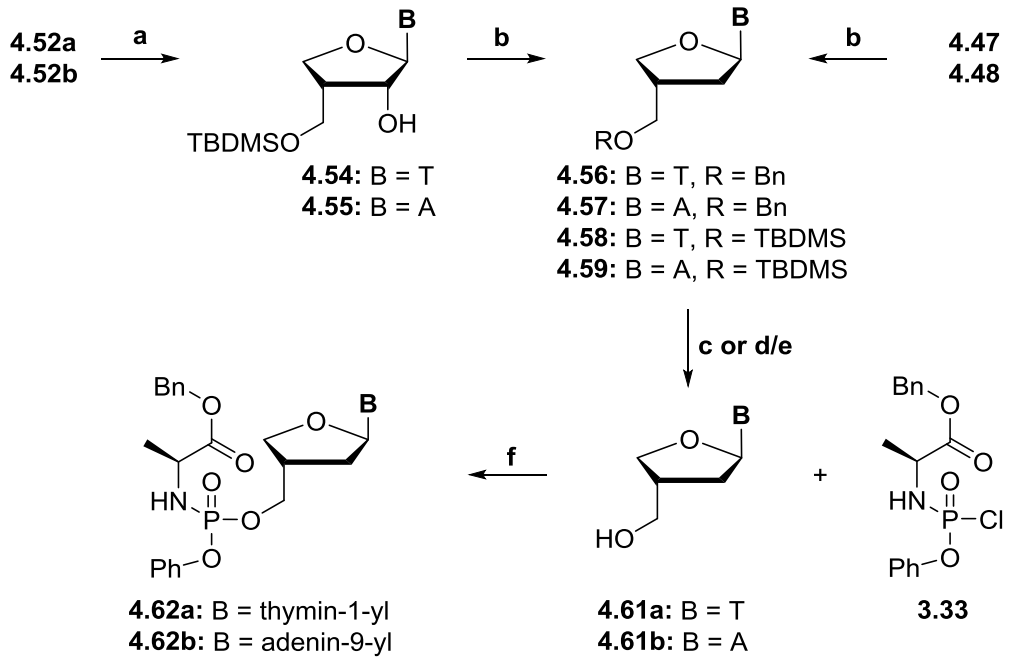
Figure 4.10. 2D ^{1}H-^{1}H NOESY spectrum of **4.41.**

Vorbrüggen coupling of the methyl anomer **4.37** and silylated thymine under microwave irradiation resulted in an inseparable mixture of two isomeric products in a 2:1 ratio.[176] Unfortunately, removal of the acetyl and benzyl protecting groups did not facilitate their separation. The ^{1}H NMR spectrum of the minor isomer showed a larger splitting of the anomeric hydrogen doublet (3.2 Hz) compared to the major compound (2.0 Hz), indicating β-oriented pyrimidine moiety **4.43** (Figure 4.8). The gHMBC confirmed the C1′-N1 attachment, while 2D NOESY ratified the relative stereochemistry. Conversely, microwave-assisted coupling between **4.37** and silylated N^6-benzoyladenine gave only the desired α-nucleoside **4.40** in 60% isolated yield. Clearly, the microwave-assisted coupling with the methyl glycoside is the method of choice to prepare the adenine nucleoside.



Scheme 4.3. Syntheses of α-D-apio-L-furanonucleosides **4.53a,b** and their 3′-deoxy counterparts **4.52a,b.** *Reagents and conditions:* (a) appropriate silylated base, 1,2-$(CH_2)_2Cl_2$, TMSOTf, rt, 4h, 85% for **4.44**; (b) appropriate silylated base, CH_3CN, 0.2 eq. TMSOTf, MW 300W, 0 °C → 150 °C (3 min), 150 °C (5 min), 40% for **4.45** and 6% for **4.46**; (c) NH_3, MeOH, rt, 4-48h, 75-96%; (d) H_2, Pd/C, MeOH, rt, overnight, 86% for **4.52a** from **4.47** and 71% for **4.53a** from **4.49**;(e) (i) $Pd(OH)_2$, HCOOH-MeOH (1:1 for **4.51**, **4.52b** from **4.48** and 1:9 for **4.53b** from **4.50**), 55 °C, 5-8h; (ii) NH_3, MeOH, rt, 3h, 80% over two steps for **4.51**, **4.52b** and **4.53b**.

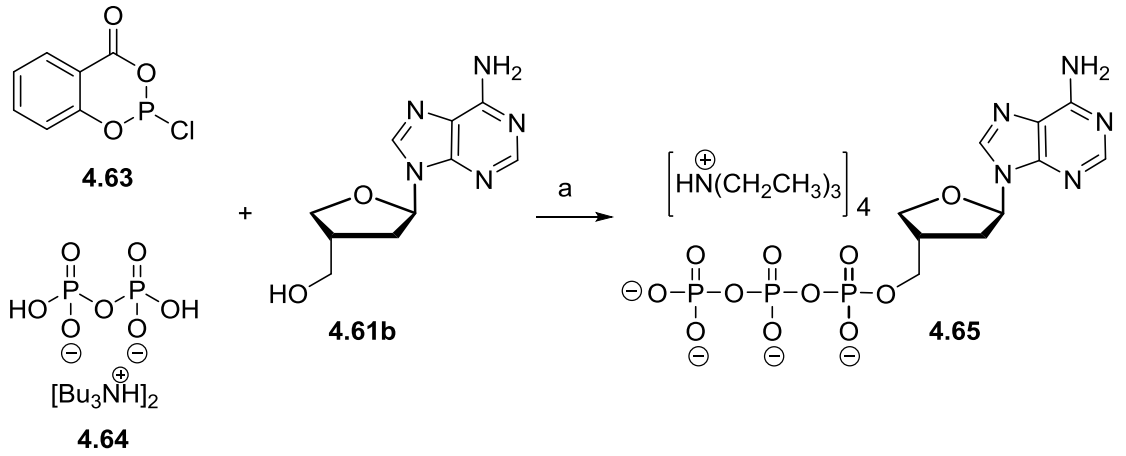
Reaction of the triacetyl apiose **4.38** with silylated thymine under classical Vorbrüggen conditions provided **4.44** in very good yield (Scheme 4.3). Microwave conditions were employed to couple **4.38** with silylated N^6-benzoyladenine to afford **4.45** and 2′-OTMS analogue **4.46** in 40% and 6% yield, respectively. The coupling products **4.39**, **4.40**, **4.44** and **4.45** were treated with ammonia in MeOH to provide the desired deacetylated products **4.47-50**. Debenzylation of thymine nucleosides **4.47** and **4.49** to give the 3′-deoxyapionucleoside **4.52a** and apionucleoside **4.53a** was realized by palladium-catalyzed hydrogenation. The same reaction condition on adenosines **4.48** and **4.50** was ineffective, as well as the use of cyclohexene and ammonium formate. This result led us to use formic acid as hydrogen source to give **4.52b** and **4.53b**. The byproduct **4.51** was converted back to **4.52b** after treatment with ammonia in MeOH.



Scheme 4.4. Synthesis of 2′,3′-dideoxy-α-D-apio-L-furanosides **4.61a,b** and their ProTides **4.62a,b**. *Reagents and conditions:* (a) TBDMSCl, imidazole, DMF, rt, overnight, 95% for **4.54** and 82 % for **4.55**; (b) (i) O-*p*-tolylchlorothiono formate, DMAP, CH_3CN, 0 °C → rt, 4h; (ii) Bu_3SnH, AIBN, toluene, reflux, 2-3h, 70-88% over two steps; (c) H_2/Pd-C, MeOH, rt, overnight, 63% from **4.56** to **4.61a**; (d) TBAF, THF, 0 °C → rt, 4h, 89% **4.58** to **4.61a**; (e) NH_4F, MeOH, 50 °C, 2 days, 87% **4.59** to **4.61b**; (f) *t*-butyl magnesium chloride, anh. THF, rt, overnight, 22% for **4.62a**; anh. NMI, anh. THF, anh. pyridine, rt, 2 days, 15% for **4.62b**.

Initially, the benzyl protected nucleosides **4.47** and **4.48** were subjected to a standard Barton- McCombie protocol to give the 2′-deoxygenated products **4.56** and **4.57** (Scheme 4.4). Different hydrogen sources were explored for the subsequent Pd-catalyzed debenzylation, but only the thymine compound **4.56** could be converted to the desired product **4.61a** with curtailed reproducibility in yield and reaction time. This was attributed to catalyst poisoning by remaining sulfur residues. Hence, we swapped to TBDMS as a protecting group to give **4.54** and **4.55** from **4.52a** and **4.52b**, respectively, in excellent yields. Compounds **4.54** and **4.55** were submitted to Barton-McCombie deoxygenation after conversion to the corresponding xanthates with *O*-*p*-tolyl chlorothionoformate in the presence of DMAP. These xanthates were isolated after a brief workup and heated in toluene with tributyltin hydride and azobisisobutyronitrile to give dideoxycompounds **4.58** and **4.59**. The TBDMS group of **4.58** and **4.59** was removed using TBAF in THF. However, the removal of tetrabutylammonium residue to get pure adenosine derivative **4.61b** was not satisfactory, hence an alternative method using NH_4F in MeOH at 55 °C for 2 days gave **4.61b** in 87% isolated yield.

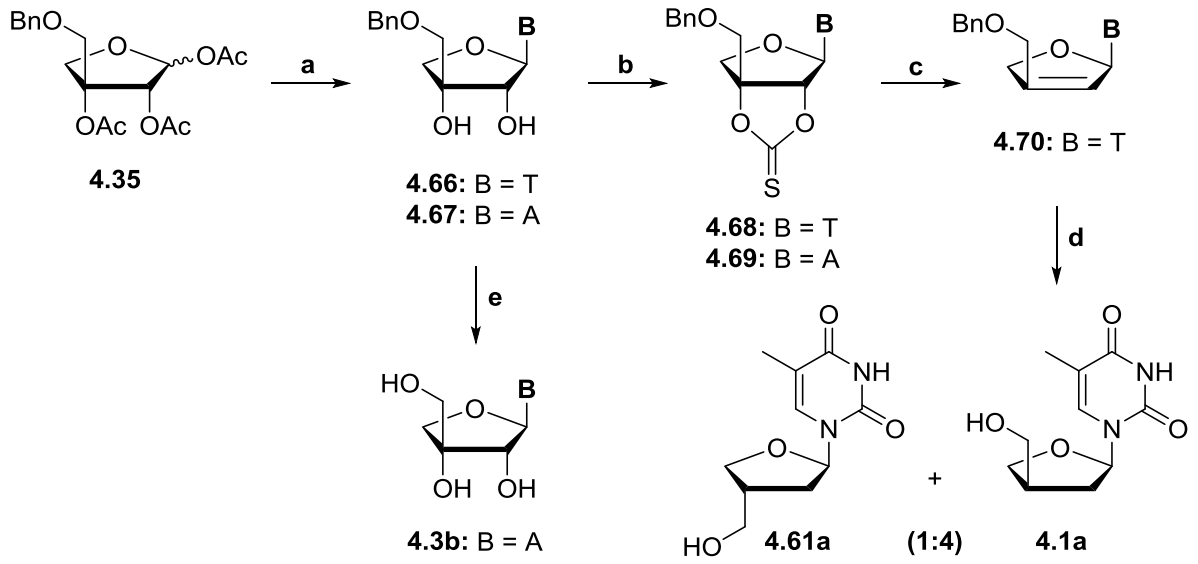
Nucleoside monophosphate prodrugs (ProTides) of compounds **4.61a** and **4.61b** were prepared using two different methods. **4.62a** was prepared by coupling **4.61a** with the phosphorochloridate **3.33**, using *tert*-butyl magnesium chloride as hydroxyl activator in anhydrous THF. On the other hand, **4.61b** was coupled with **3.33** to provide **4.62b**, using in this case N-methylimidazole as a base in a mixture of anhydrous THF and pyridine as solvents. In both cases, the desired compounds were obtained as a mixture of two diasteroisomers resulting from the two possible configurations of the phosphorous stereo center, as confirmed by the presence of two equal height peaks in the ^{31}P NMR spectrum. Moreover, in order to study the effectiveness of **4.61b** as a potential HIV RT inhibitor, the 2′,3′-dideoxy-α-D-apio-L-furanonucleoside **4.61b** was also converted to its corresponding triphosphate **4.65** following the method of Caton-Williams (Scheme 4.5)[177].



Scheme 4.5. Synthesis of 2′,3′-dideoxy-α-D-apio-L-furanoadenosine triphosphate **4.65**. *Reagents and conditions:* (a) (i) **4.63**, **4.64**, Bu_3N, anh. DMF, rt, 1.5h; (ii) **4.61b**, anh. DMF, rt, 1.5h; (iii) I_2 (3% in 9:1 Py-H_2O), rt, 20 min, H_2O, rt, 1.5h, 48% over three steps.

4.3.1.2. Syntheses of β-D-apio-D-furanonucleosides

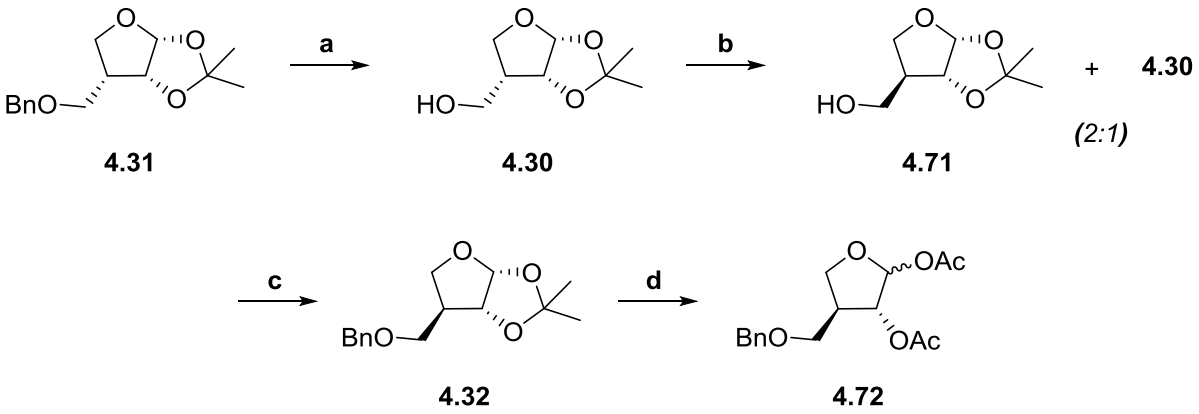
Having established that the nucleosides synthesized in section 4.4.1.1 are all L-furanosides, we envisaged easy access to 2′,3′-dideoxy-β-D-apio-D-furanose nucleosides **4.1a**,**b** involving Corey-Winter olefination and stereoselective hydrogenation as key steps (Scheme 4.6). During catalytic hydrogenation the *syn*-addition of the hydrogen atoms to the double bond is anticipated to occur from the face opposite to the one containing the nucleobase.[178] A combination of classical and microwave assisted Vorbrüggen coupling on **4.35**, followed by deprotection and subsequent thiocarbonyl imidazole treatment provided **4.68** and **4.69** for Corey-Winter olefination. Unfortunately, the adenine derivative degraded in trimethylphosphite at 120 °C. The thymine derivative did gave the desired product **4.70** in excellent yields but the hydrogenation reaction resulted in a mixture of diastereomers **4.1a** and **4.61a** that are inseparable by column chromatography. This forced us to find an alternate method to prepare the target molecules.



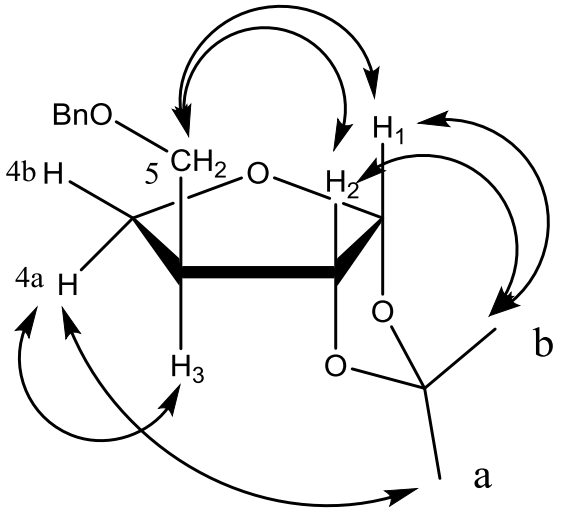
Scheme 4.6. Synthesis of β-D-apio-D-furanonucleosides **4.3b** and dideoxy congener **4.1a.** *Reagents and conditions:* (a) (i) silylated thymine, 1,2-$(CH_2)_2Cl_2$, TMSOTf, rt, 4h, for **4.66** or silylated N^6-BzA, CH_3CN, 0.2 eq. TMSOTf, MW 300W, 0 °C → 150 °C (3 min), 150 °C (5 min), for **4.67** (ii) 7N NH_3-MeOH, rt, 4-48h, 97% for **4.66** and 46% for **4.67** over two steps; (b) thiocarbonyl diimidazole, DMF, 80 °C, 90 min, 89% for **4.68** and 78% for **4.69**; (c) $P(OCH_3)_3$, 120 °C, 6h, 90 %; (d) H_2, Pd/C, MeOH, rt, 4h, 89%; (e) (i) $Pd(OH)_2$, HCOOH-MeOH (1:4), 55 °C, 5h (ii) NH_3, MeOH, rt, 3h, 89%.

Carey and co-workers found that 1,2-*O*-isopropylidene-D-apio-L-furanose (**4.8**) equilibrates into the D- and L-furanose form in acidic acetone (section 4.3, Method-1), which inspired us to use similar conditions for the epimerization of **4.30**. We hypothesized that the absence of the 3-hydroxyl group would eliminate the repulsive dipole interaction with oxygen at position 2, while the steric interaction of the hydroxymethyl group with the 2-oxygen could result in a favorable D-furano isomer ratio. Hence compound **4.31** was debenzylated and then treated with acetone-conc. H_2SO_4 to afford the desired epimer **4.71** in 73% yield (Scheme 4.7). It is noteworthy that 3 equilibrium cycles were required to convert most of the starting material to the desired D-furano epimer. Benzylation of the compound **4.71** gave **4.32** and the structure of this was confirmed by 2D ^{1}H-^{1}H NOESY experiment (Figure 4.11 and 4.12). The following nOe cross-peaks confirmed the stereochemistry: (a) both the anomeric (δ_{ppm} 5.79) and the C-2 (δ_{ppm} 4.56) protons give nOe's with 3-CH_2 (δ_{ppm} 3.37); (b) 3-H (δ_{ppm} 2.56) interacts strongly with 4-Hα (δ_{ppm} 4.09) and only weakly

with 4-Hβ (δ_{ppm} 3.83). Hydrolysis and acetylation of this isomer rendered 3-deoxy-D-apio-D-furanose derivative **4.72** in good yields.



Scheme 4.7. Synthesis of D-apio-D-furanose coupling partner **4.72** *via* differential cyclization. *Reagents and conditions:* (a) H_2, Pd/C, MeOH, rt, 5h, 90%; (b) acetone, conc. H_2SO_4, rt, 1.5h, Na_2CO_3, 45 min, 73% (after 3 cycles); (c) DMF, NaH, 0°C, 10 min, BnBr, 0°C → rt, 18h, 95%; (d) (i) 80% aq. AcOH, 80 °C, 18h; (ii) pyridine, Ac_2O, rt, 18h, 79%.



Compound **4.32**

Figure 4.11. Notable nOe interactions observed for compound **4.32**

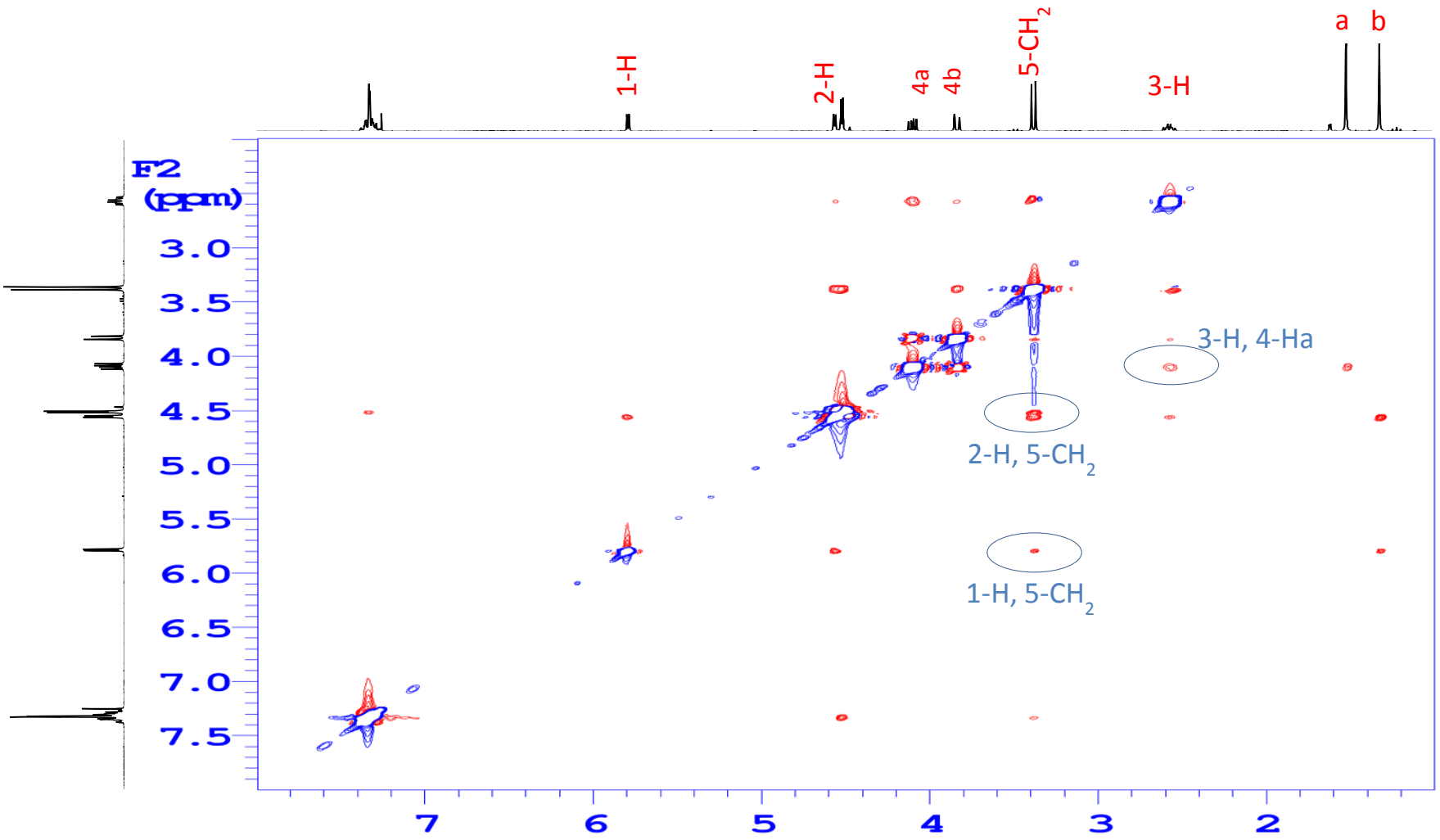
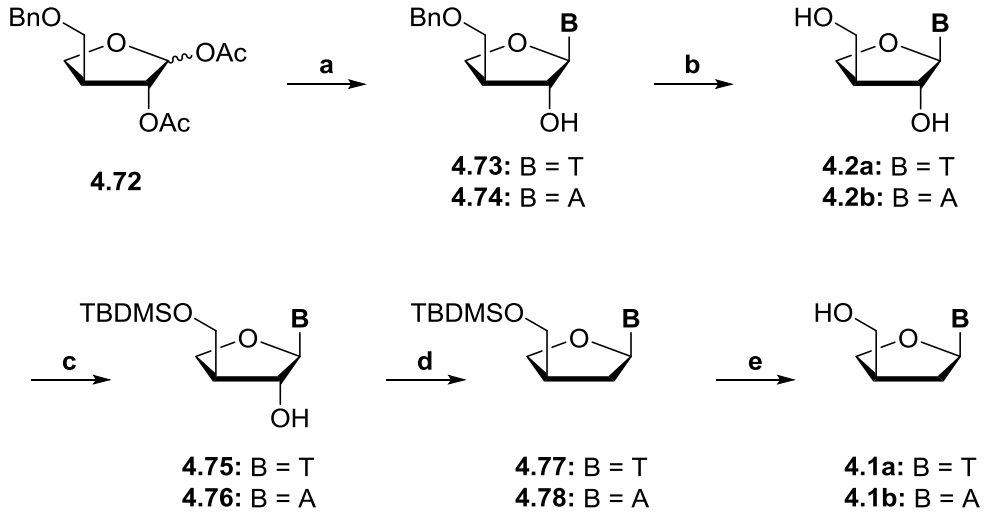


Figure 4.12. 2D ^{1}H-^{1}H NOESY spectrum of compound **4.32**

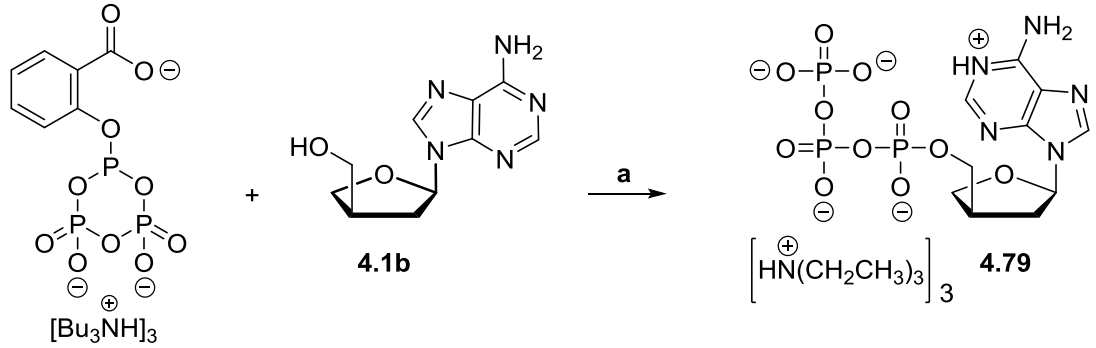
Having the glycosyl donor **4.72** at hand, coupling with thymine and benzoyladenine towards the intended products **4.2a**,**b** and **4.1a**,**b** was performed as before (Scheme 4.8). It is noteworthy that, compared to the L- furano series, Vorbrüggen reaction generally gave lower yields of the coupling products. Moreover, coupling of **4.72** with silylated benzoyladenine produced significant amount of α-isomer (11%) with only 28% of desired β-nucleoside.



Scheme 4.8. Syntheses of 3′-deoxy-β-D-apio-D-furanonucleosides **4.2a,b** and their 2′,3′-dideoxy counterparts **4.1a,b.** *Reagents and conditions:* (a) (i) silylated thymine, 1,2-$(CH_2)_2Cl_2$, TMSOTf, rt, 4h, or silylated N^6-BzA, CH_3CN, 0.2 eq. TMSOTf, MW 300W, 0 °C → 150 °C (3 min), 150 °C (5 min); (ii) NH_3, MeOH, rt, 4-48h, 60% for **4.73**, 28% of **4.74** and 11% of its α-anomer;(b) H_2, Pd/C, MeOH, rt, overnight, 86% for **4.2a** or (i) $Pd(OH)_2$, HCOOH-MeOH (1:4), 18h; (ii) NH_3, MeOH, rt, 3h, 88% over two steps for **4.2b**; (c) TBDMSCl, imidazole, DMF, rt, 18h, 88% for **4.75** and 82% for **4.76**; (d) (i) O-*p*-tolylchlorothiono formate, DMAP, CH_3CN, 0 °C → rt, 4h; (ii) Bu_3SnH, AIBN, toluene, reflux, 2-3h, 90% for **4.77** and 81% for **4.78** over two steps; (e) NH_4F, MeOH, 50 °C, 2 days, 86% for **4.1a** and 94% for **4.1b**.

Compounds **4.1** are reported to be inactive against viruses. To examine the HIV inhibition potential of a monophosphate prodrug, **4.1b** was converted to its triphosphate **4.79** (Scheme 4.9). The reaction of Van Boom's reagent (**4.63**) and pyrophosphate (**4.64**) resulted in a cyclic diphosphophosphite salicylate, which was reacted with **4.1b** followed by oxidation and hydrolysis using iodine/H_2O gave triphsophate **4.79**.[177] The yield of this conversion was lower than observed for the L-

furano isomer. ^{1}H NMR indicated the formation of an internal salt. The ^{31}P NMR of this compound is uncharacteristic of triphosphate salts, as it showed two broad peaks (figure 4.13, red). The addition of 2 equivalents of triethylamine disrupted this internal salt leading to the appearance of the characteristic triphosphate peaks (figure 4.13, blue).



Scheme 4.9. Synthesis of 2′,3′-dideoxy-β-D-apio-D-furanoadenosine triphosphate **4.79**. *Reagents and conditions:* (a) (i) **4.63**, **4.64**, Bu_3N, anh. DMF, rt, 1.5h; (ii) **4.61b**, anh. DMF, rt, 1.5h; (iii) I_2 (3% in 9:1 Py-H_2O), rt, 20 min, H_2O, rt, 1.5h, 21% over three steps.

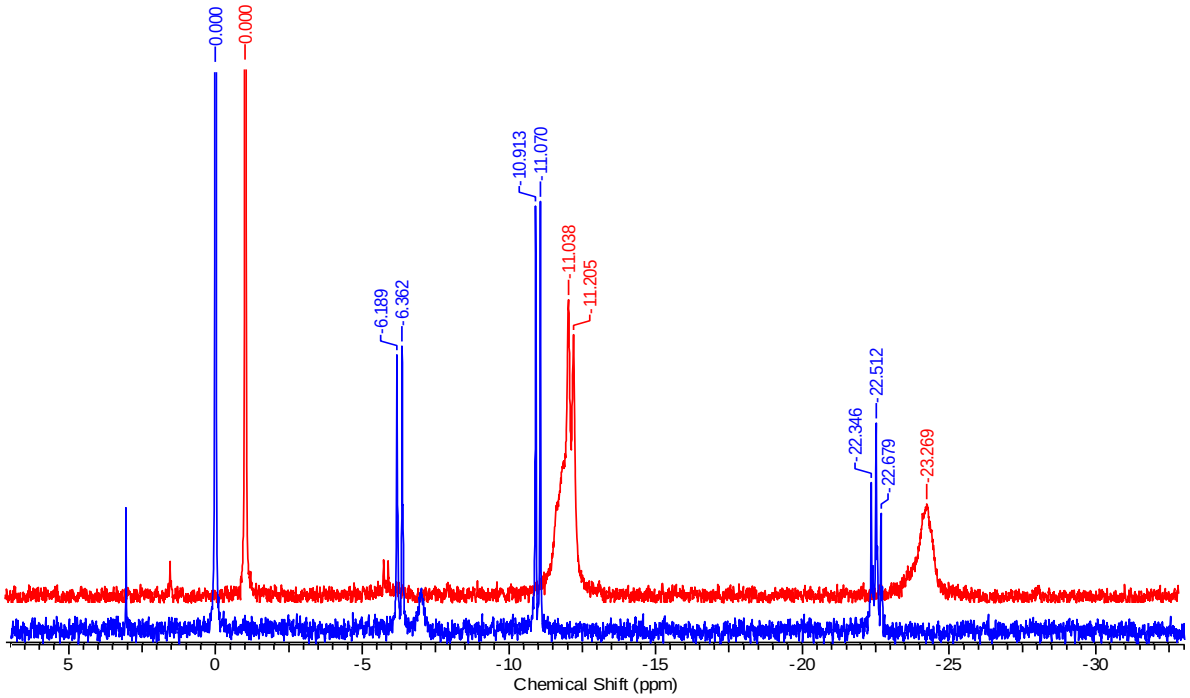
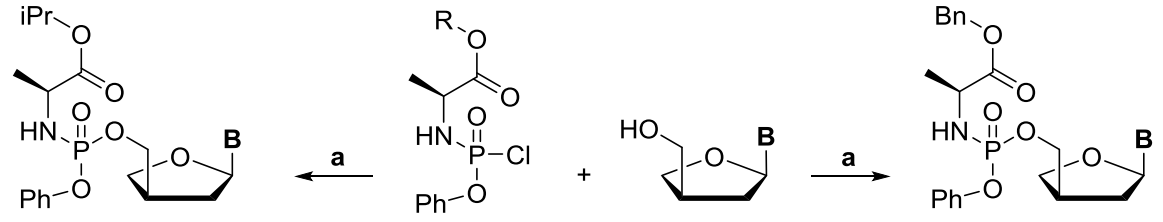


Figure 4.13. ^{31}P-NMR of **4.79** before (red) and after triethylamine treatment (blue).

The benzyl/isopropyl alaninyl phosphoramidate prodrugs of the 2′,3′-dideoxy-β-D-apio-D-furanonucleoside **4.1a**,**b** were synthsized by reacting the latter with the appropriate phosphochloridate (**3.33/4.60**) using slightly modified reaction condition employed for the synthesis of **4.62b**. A prepTLC purification of the reaction mixture gave ProTides **4.6a**,**b** and **4.80a**,**b** in low to excellent yields (Scheme 4.10).



Scheme 4.10. Synthesis of 2′,3′-dideoxy-β-D-apio-D-furanonucleoside ProTides **4.6a**,**b** and **4.80a**,**b**. *Reagents and conditions:* (a) anh. NMI, anh. THF, anh. pyridine, rt, 2 days, 26-88% .

4.3.2. Pharmacological evaluation

4.3.2.1. Enzymatic assay using carboxypeptidase Y

The putative mechanism of activation of ProTides (Figure 4.14, 4.16) involves an enzymatic cleavage of the ester (a) mediated by an esterase- or carboxypeptidase-type enzyme followed by spontaneous cyclisation to release the aryl moiety (b) and to open the unstable mixed anhydride ring by water (c) providing the intermediate metabolite **4.83**. The cleavage of the phosphorous-nitrogen bond (d) requires a phosphoramidase type enzyme, perhaps related to human HINT-1, to release the monophosphate form (**4.84**). In order to investigate the bioactivation of L-furano ProTides **4.62a,b** and D-furano ProTide **4.6a**, these compounds were incubated with carboxypeptidase Y enzyme in acetone-d_6 and Trizma buffer (pH = 7.6) recording a ^{31}P NMR at specific intervals.

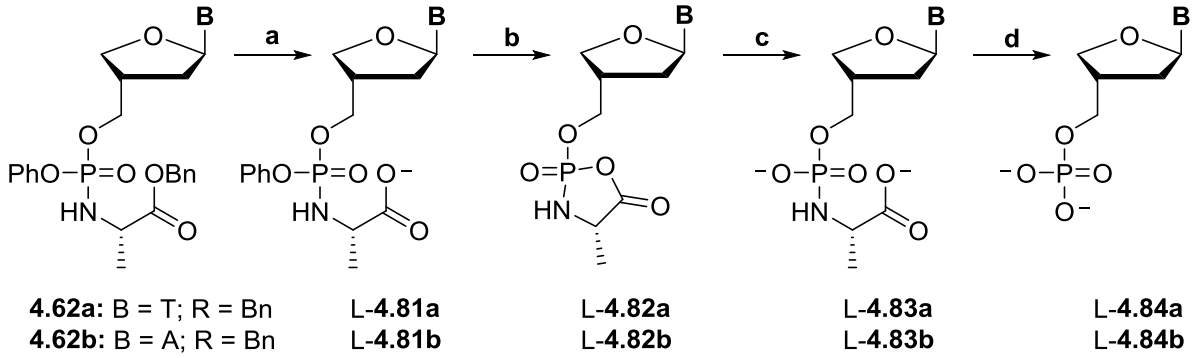


Figure 4.14. Putative mechanism of bioactivation for L-furano ProTides.

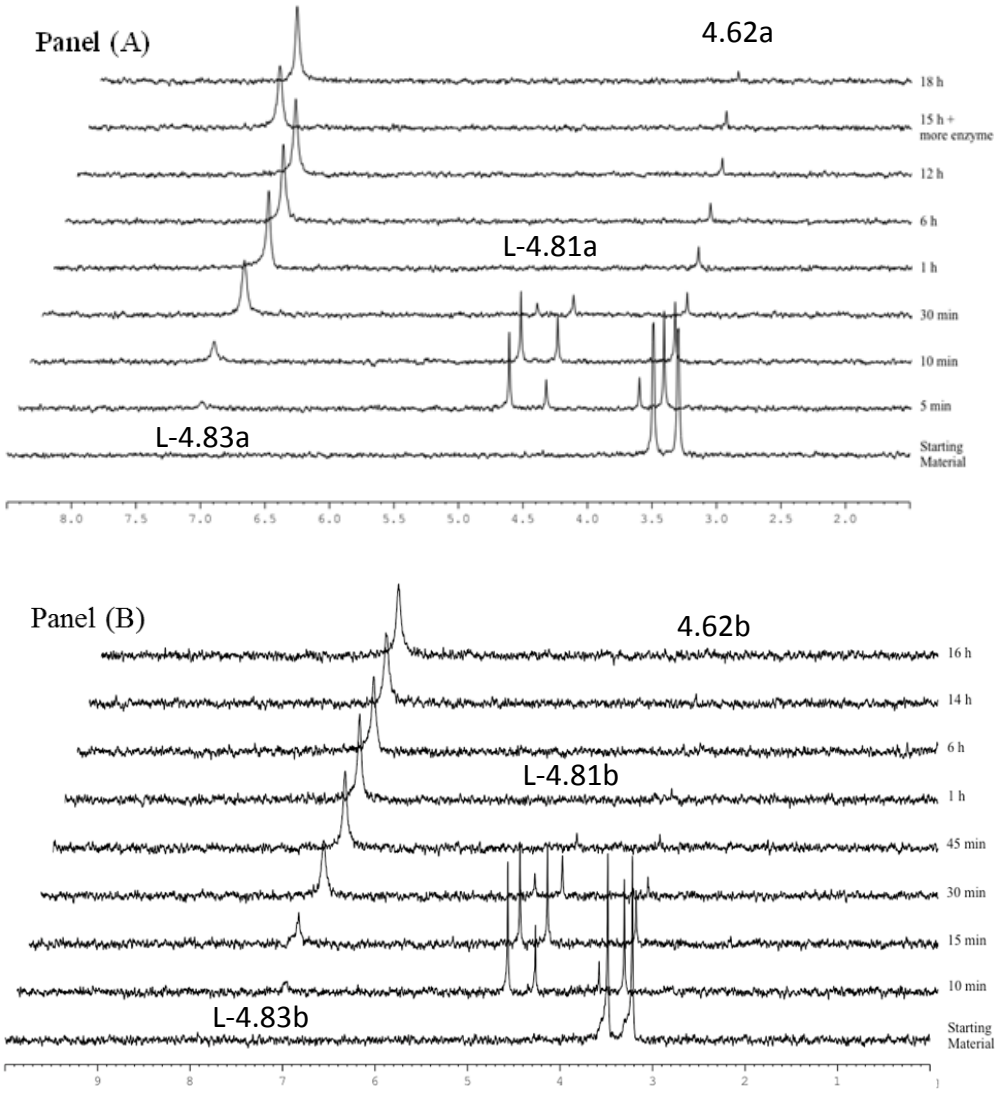


Figure 4.15. Bioactivation study for L-furano compounds **4.62a** (Panel A) and **4.62b** (Panel B) using carboxypeptidase Y enzyme.

Compound **4.62a** (^{31}P NMR = 3.3 and 3.5 ppm, Figure 4.15a) showed a fast conversion to the intermediate metabolite L-**4.81a** (^{31}P NMR = 4.7 and 4.4 ppm) with its formation within 5 minutes after the addition of the enzyme, which is then converted to compound L-**4.83a** (^{31}P NMR = ~7.0 ppm). Interestingly, one of the diastereoisomers (^{31}P NMR = 3.3 ppm) seems to be more slowly converted compared to the other. In fact, after 18h, it is still present, even after the addition of an extra portion of enzyme, while the diastereomer at 3.5 ppm appears fully converted after about 10 minutes. In contrast, compound **4.62b** (^{31}P NMR = 3.2 and 3.4 ppm, Figure 4.15b) shows a near complete conversion of both diasteroisomers to the metabolite L-**4.83a** (^{31}P NMR = ~7.0 ppm) through the intermediate L-**4.81a** (^{31}P NMR = ~4.5 ppm) after 1 hour, although there again exists a clear difference in kinetics. From this study it thus appears that both compounds are rapidly converted to the intermediate metabolite **4.83**, with a slightly better conversion rate for the adenine derivative compared to the thymine derivative.

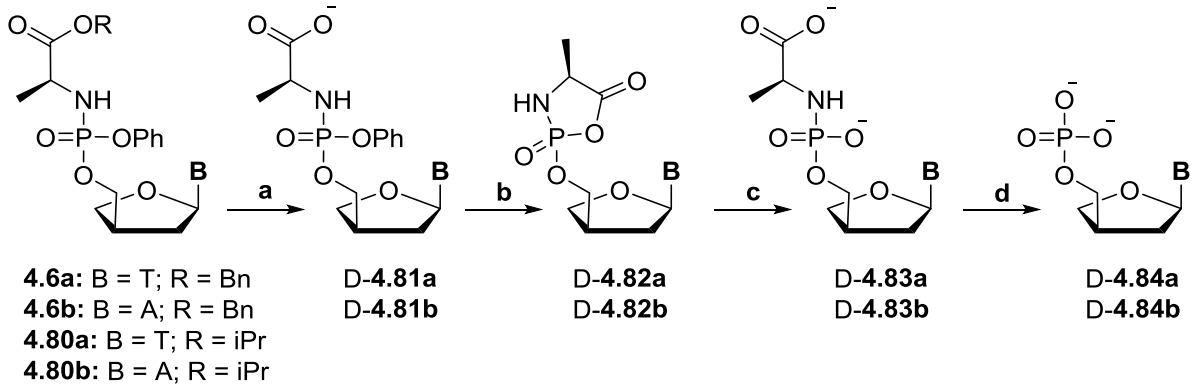


Figure 4.16. Putative mechanism of bioactivation for D-furano ProTides.

Both diastereoisomers of the D-furano ProTide **4.6a** were processed to intermediate D-**4.82a** within 20 minutes followed by the formation of phosphoramide D-**4.83a** within an hour. From figure 4.17 it is evident that **4.6a** lacked pronounced diastereomeric discrimination by carboxypeptidase enzyme. Following the trend for adenine analogue **4.62b**, we assume that **4.6b** would be processed at the least with the rate of thymine analogue **4.6a**.

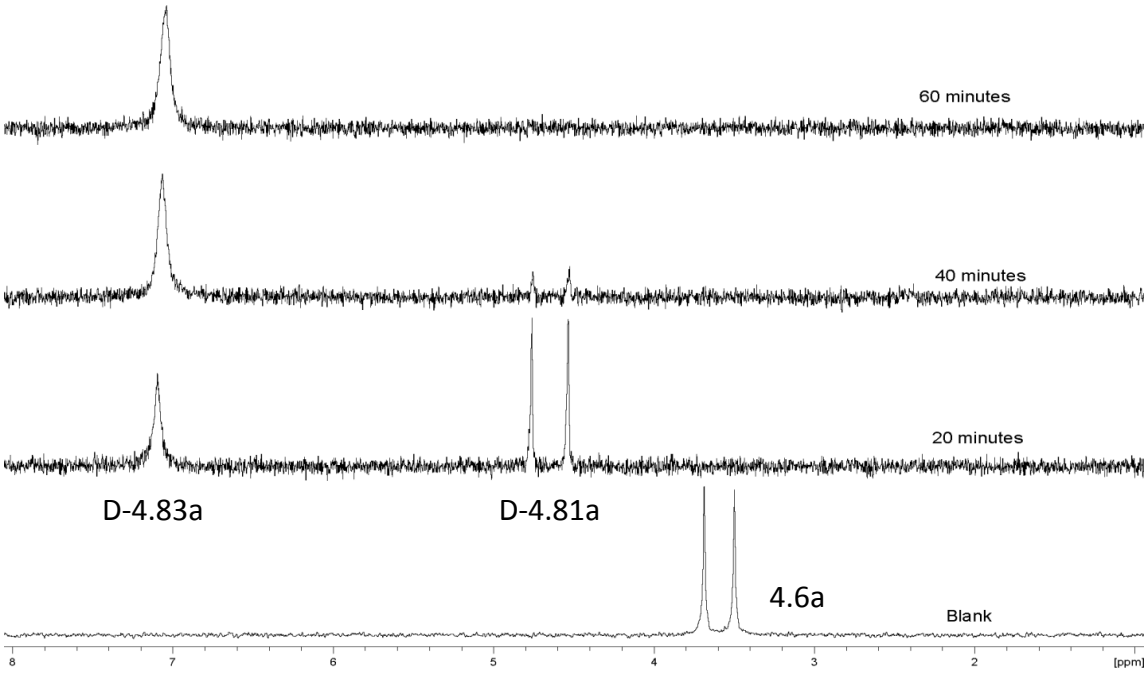


Figure 4.17. Bioactivation study for D-furano compound **4.6a** using carboxypeptidase Y enzyme.

4.3.2.2. DNA chain termination study using HIV Reverse Transcriptase

Another prerequisite for ProTides to show good anti-HIV activity is that their corresponding triphosphates are good alternative substrates for RT. Hence, the ability of triphosphates **4.65**/**79** to act as a substrate of HIV-RT was investigated in a primer-template assay.[43] The template has overhanging T residues to test incorporation of the modified A nucleotide. Figure 4.18 clearly shows that both nucleotides **4.65** and **4.79** function as chain terminators. The D-furano analogue **4.79** is more efficiently incorporated than **4.65**, but compared to natural substrate dATP, requires a higher concentration and longer time for complete incorporation. The characteristics of **4.79** towards HIV RT render the corresponding ProTides potentially useful HIV inhibitors.

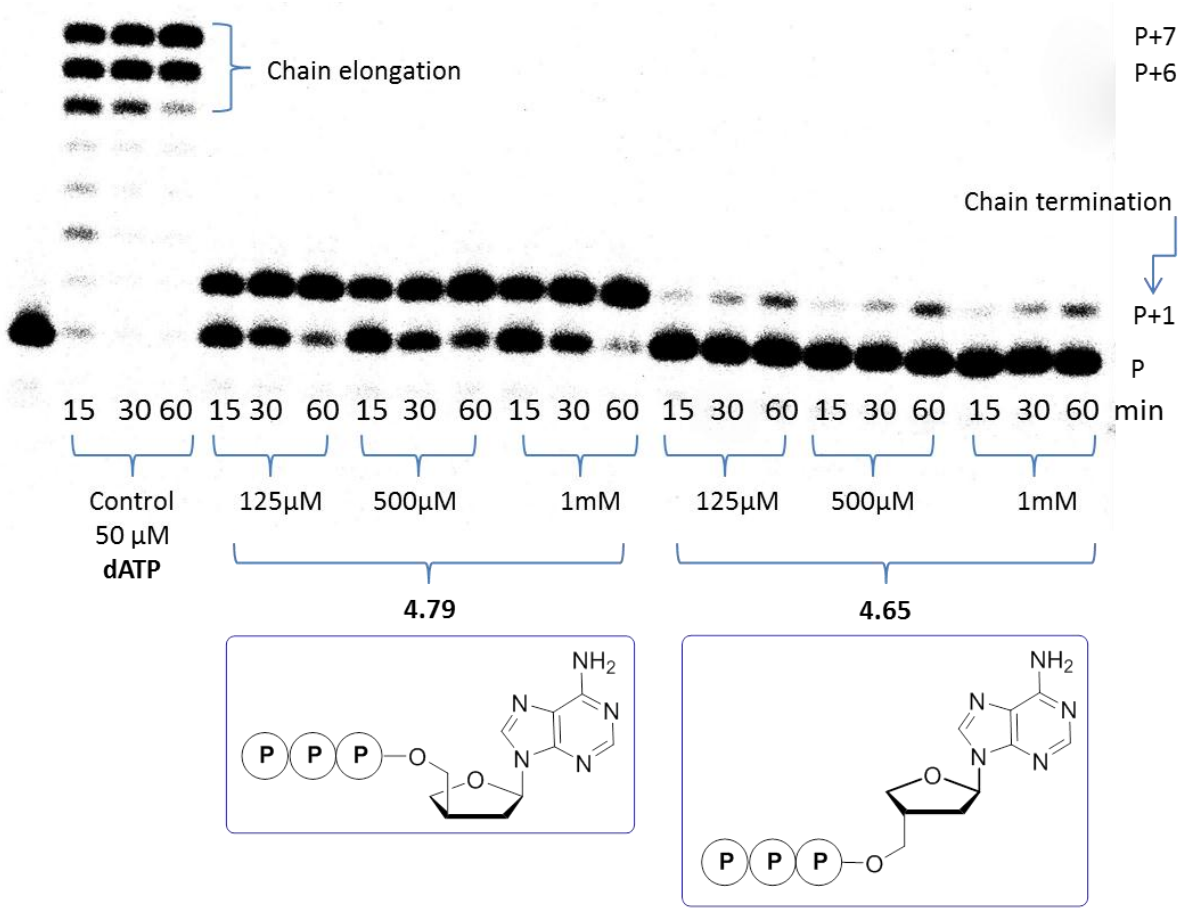


Figure 4.18. Chain termination through incorporation of **4.65** and **4.79** by HIV RT. The DNA polymerization mixtures containing 125 nM annealed (labeled) primer-template complex, were treated with 125, 500, or 1000 µM of modified triphosphate (**4.65/ 4.79**) and 0.03 $U.\mu l^{-1}$ HIV RT and incubated at 37°C. Aliquots were taken after 15, 30 and 60 min. In the control reaction, 50 µM of natural dATP was used. Samples were separated on a 0.4 mm 20% denaturing polyacrylamide gel and the bands visualized using phosphorimaging.

4.3.2.3. Antiviral and other data

2′,3′-Dideoxy-β-D-apio-D-furanoadenosine phosphoramidate ProTides **4.6b** and **4.80b** combine potent anti-HIV activity with reasonable selectivity (Table 4.3). The benzylester **4.6b** exhibits anti-HIV activity comparable or superior to the acyclic nucleoside phosphonate PMPA, but are 10-100 fold less potent than nucleoside analogues in therapeutic use. The ProTides **4.6a**,**b**, **4.62a**,**b** and **4.80a**,**b** are weak to moderate inhibitors of murine leukemia (L1210), human T-lymphocyte (CEM) and human cervix carcinoma (HeLa) cell proliferation (Table 4.4). Compound **4.61a**

inhibited 1 µM dThd phosphorylation by the following thymidine kinases: TK-2 (IC_{50}: 150±78 µM), HSV-1 TK (IC_{50}: 117±52 µM) and VZV TK (IC_{50}: 271±59 µM) and caused moderate inhibition of *M. tuberculosis* thymidylate kinase (K_i: 48 µM).

Table 4.3. Anti-HIV property of ProTides **4.6a**,**b** and **4.80a**,**b**

	EC_{50} in MT-4 cells (µM)					*EC_{50} in CEM cells (µM)*	
	HIV-1 *(NL4.3)*		***HIV-2*** *(ROD)*		***CC_{50}***	***HIV-1*** *(IIIb)*	***HIV-2*** *(ROD)*
4.6a	*>250*	-	*>250*	-	*196*	-	-
4.80a	*>250*	-	*>250*	-	*>250*	-	-
4.6b	*0.5*	*0.5*	*1*	*1*	*93*	*0.5*	*1.5*
4.80b	*26*	*2*	*27*	*21.5*	*>250*	*7.5*	*38*
PMPA	*1.5*	*2*	*1*	*1*	*>250*	*3*	*2.5*

'-' = not performed

Table 4.4. Inhibitory effects of compounds on the proliferation of cancer [a]

	L1210	*CEM*	*HeLa*
4.6a	*113 ± 21*	*108 ± 11*	*159 ± 32*
4.6b	*110 ± 17*	*80 ± 4*	*53 ± 11*
4.62a	*167 ± 85*	*113 ± 3*	*177 ± 103*
4.62b	*79 ± 4*	*73 ± 5*	*173 ± 58*
4.80a	*> 250*	*> 250*	*> 250*
4.80b	*226 ± 35*	*204 ± 3*	*≥ 250*

[a] *IC_{50} in µM, murine leukemia cells (L1210/0), human T-lymphocyte cells (CEM/0) and human cervix carcinoma cells (HeLa)*

The L-furano 3′-deoxyapiose nucleosides **4.52a**,**b** and dideoxynucleosides **4.61a**,**b** failed to show significant inhibitory activity when tested against HIV-1,2 and panel of viruses or cytotoxicity in cell culture. Likewise, the 3′-deoxy-β-D-apio-D-furano nucleosides **4.2a**,**b** and dideoxynucleosides **4.1a**,**b** also lacked activity against HIV-1,2 and cancer proliferation. The inactivity of thymine analogue prodrugs **4.6a** and **4.80a** could be due to inefficient metabolism to corresponding monophosphate by HINT-1 type phosphoramidase enzyme, or the apioNMP/NDP is a poor substrate for the corresponding NMPKs/NDPKs. Alternatively, if efficiently activated to the

corresponding 2′,3′-dideoxy-β-D-apio-D-furanothymidine triphosphate, the latter may be inefficiently incorporated by HIV RT.

4.4. Conclusions

A synthetic methodology was developed to create a family of D-apio-D- and L-furano nucleosides, their 3′-deoxy- and 2′,3′-dideoxy-analogues. The latter were also converted to some representative ProTides. The 2′,3′-dideoxy-β-D-apio-D-furanoadenosine phosphoramidate prodrugs showed promising activity against HIV-1,2. These results are in line with the observation that the triphosphate of 2′,3′-dideoxy-β-D-apio-D-furanoadenosine is readily accepted by HIV RT and acts as a DNA chain terminator. The other nucleosides or their prodrugs were neither toxic nor active against a panel of viruses and cancer cell lines.

4.5. Experimental Section

4.5.1. Synthesis

All reagents were from standard commercial sources and of analytic grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. All reactions were carried out under argon atmosphere using dry solvents, unless specified otherwise. Room temperature or rt referes to 25±5 °C. Precoated Merck silica-gel F254 plates were used for TLC. The spots were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid-anisaldehyde spray or CAM spray. Column chromatography was performed on silica gel (40-63 μm, 60 Å) or on Reveleris flash chromatography system. NMR spectra were recorded on a Varian Mercury 300 MHz or a Bruker Avance II 700 MHz spectrometer. Chemical shifts are given in ppm (δ), calibrated to the residual solvent signals or TMS. Exact mass measurements were performed on aWaters LCT PremierXETMTime of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and

modular LockSpray TM interface. Samples were infused in a CH_3CN/H_2O (1:1v/v) mixture at 10 mL/min. The microwave reactions were carried out in Milestone MicroSYNTH Advanced Microwave Synthesis Labstation, equipped with 2 X 800 W magnetrons (effective maximum output 1500W pulsed/continuous), an optical fiber temperature sensor, a pressure sensor, in continues power mode in a closed PTFE vessel. For nucleosides, NMR signals of sugar protons and carbons are indicated with a prime, and signals of base protons and carbons are given without a prime.

3-Iodo-1,2-*O*-isopropylidene-α-D-erythrofuranose (4.33): Compound **3.1** (500 mg, 3.1 mmol), triphenylphosphine (1.22 g, 4.65 mmol) and imidazole (316 mg, 4.65 mmol) was dissolved in anhydrous toluene (25 mL) under inert atmosphere. To this at 115 °C was added iodine (945 mg, 3.72 mmol) portion-wise and the mixture was stirred at this temperature for 3h. After the reaction mixture has cooled, 10% aq.$Na_2S_2O_3$ was added. The products extracted in ethylacetate, dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography using 5-10% ethylacetate in hexanes to afford title compound **4.33** (190 mg, 22%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.31 (s, 3H, CH_3), 1.49 (s, 4H, CH_3), 3.74 - 3.92 (m, 2H, 3,4-H), 3.95 - 4.03 (m, 1H, 4-H), 4.52 (t, *J* = 3.66 Hz, 1H, 2-H), 5.74 (d, *J* = 3.51 Hz, 1H, 1-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 17.21 (3-C), 25.34 (*C*H_3), 25.44 (*C*H_3), 71.35 (4-C), 79.03 (2-C), 102.84 (1-C), 110.78 (*C*$(CH_3)_2$).

3-Oxo-1,2-*O*-isopropylidene-α-D-erythrofuranose (4.11): To a solution of compound **3.1** (1.0 g, 6.24 mmol) in anhydrous CH_2Cl_2 (12.5 mL) was added bis-acetoxyiodobenzene (BAIB, 2.41 g, 7.5 mmol) followed by (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO, 195 mg, 1.25 mmol) at room temperature under an argon atmosphere. The mixture was stirred at room temperature for 4h. The contents of the reaction was directly loaded on silica-gel and eluted with 30% EtOAc-hexanes to afford pure product **4.11** (890 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.35 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 4.03 (dd, *J* = 4.06, 17.57 Hz, 1H, 4-H), 4.29 (s, 1H, 2-H), 4.32 (dd, *J* = 0.59, 17.57 Hz, 1H, 4-H), 6.02 (d, *J* = 4.39 Hz, 1H, 1-H).

1,2-*O*-Isopropylidene-5-(*O*-benzyl)-α-D-apio-D-furanose (4.13): To a stirring solution of benzyloxymethyltributlytin ($BOMSnBu_3$, 5.93 g, 14.4 mmol) in dry THF (35 mL) at -78 °C under inert condition, was added dropwise *n*-butyllithium (1.6M in hexanes,19.5 mL, 31.3 mmol) and stirred for additional 1h. To this mixture was then added dropwise a solution of compound **4.11** (1.9 g, 12.02 mmol) in 10 mL THF and stirred at -78 °C for 3h. The reaction was quenched with saturated NH_4Cl solution and by vigorous stirring. EtOAc (100 mL) was then added to facilitate the layer separation. Organic layer was separated and the aqueous layer was extracted twice with EtOAc (50 mL). Combined organic layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography eluting with 17% EtOAc-hexanes to afford **4.13** (2.3 g, 68%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.37 (s, 3H, CH_3b), 1.58 (s, 3H, CH_3a), 2.85 (s, 1H, 3-OH), 3.46 (d, *J* = 10.25 Hz, 1H, 5-H), 3.56 (d, *J* = 10.25 Hz, 1H, 5-H), 3.71 (d, *J* = 9.08 Hz, 1H, 4-Ha), 3.80 (d, *J* = 9.08 Hz, 1H, 4-Hb), 4.39 (d, *J* = 3.81 Hz, 1H, 2-H), 4.54 - 4.71 (m, 2H, C*H*$_2$Ph), 5.76 (d, *J* = 4.10 Hz, 1H, 1-H), 7.27 - 7.40 (m, 5H, CH_2*Ph*).

1,2,3-Tri-(*O*-acetyl)-5-(*O*-benzyl)-α/β-D-apio-D-furanose (4.35): A solution of **4.13** (2.5 g, 8.92 mmol) in 80% aq. acetic acid (25 mL) was stirred at 80 °C for 8h. The reaction mixture was evaporated to give the crude intermediate as syrup. This syrup was dissolved in pyridine (20 mL) and DMAP was added (100 mg) followed by acetic anhydride (10 mL, 106 mmol). The solution was stirred at 55 °C for 16h. Then, the solvent was removed under vacuum and the resulting residue was partitioned between EtOAc and water. Organic layer separated, combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was purified by silica-gel column chromatography (15-20% EtOAc-hexanes) to yield **4.35** (2.45 g, 75%) as a colorless oil as a mixture of α + β isomers (2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.96 (s, 3H, major), 2.08 (s, 3H, major), 2.08 (s, 2H, minor), 2.09 (s, 1H, minor), 2.10 (s, 3H, major), 3.75 (d, *J* = 9.67 Hz, 0.47H, minor), 3.89 (d, *J* = 10.54 Hz, 1H, major), 3.96 (d, *J* = 9.67 Hz, 0.5H, minor), 4.05 (d, *J* = 10.54 Hz, 1H, major), 4.22 (d, *J* = 10.25 Hz, 1H, major), 4.26 (d, *J* = 10.54 Hz, 0.52H, minor), 4.32 (d, *J* = 10.54 Hz, 0.5H, minor), 4.34 (d, *J* = 10.25 Hz, 1H, major), 4.51 - 4.62 (m, 3H, major & minor),

5.42 (d, *J* = 4.69 Hz, 0.44H, minor), 5.49 (d, *J* = 1.17 Hz, 1H, major), 6.08 (d, *J* = 1.17 Hz, 1H, major), 6.33 (d, *J* = 4.69 Hz, 0.43H, minor), 7.27 - 7.41 (m, 7H, major & minor). ESI-HRMS $[M+Na]^+$ calcd, 389.1212; found, 389.1242.

1,2-*O*-Isopropylidene-3-deoxy-5-(*O*-benzyl)-β-D-apio-L-furanose (4.31): To a solution of **4.13** (3.5 g, 12.5 mmol) in dry THF (75 mL) was added NaH (60% in mineral oil, 1.5 g, 37.45 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 1h. To this mixture was slowly added CS_2 (11.2 mL, 188 mmol) and MeI (24.0 mL, 375 mmol) and stirred at room temperature for 1h. The reaction mixture was evaporated to give crude xanthate. The xanthate was suspended in dry toluene (75 mL), triethylborane (19.0 mL, 19.0 mmol, 1.0 M solution in THF) and *n*-Bu_3SnH (5 mL, 19.0 mmol) were added at room temperature and the mixture was stirred for further 3h. The reaction mixture was quenched with water, extracted with EtOAc, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (10% EtOAc-hexanes) to give **4.31** (2.26 g, 68 %) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32 (s, 3H, CH_3a), 1.49 (s, 3H, CH_3b), 2.37 - 2.52 (m, 1H, 3-H), 3.52 (dd, *J* = 9.23, 7.47 Hz, 1H, 5-H), 3.69 (dd, *J* = 11.28, 8.35 Hz, 1H, 4-Hb), 3.78 (dd, *J* = 9.37, 6.74 Hz, 1H, 5-H), 4.01 (dd, *J* = 8.35, 7.18 Hz, 1H, 4-Ha), 4.46 - 4.60 (m, 2H, C*H*$_2$Ph), 4.65 (t, *J* = 4.10 Hz, 1H, 2-H), 5.83 (d, *J* = 3.81 Hz, 1H, 1-H), 7.24 - 7.42 (m, 5H, CH_2*Ph*).

1,2-Di-*O*-acetyl-3-deoxy-5-(*O*-benzyl)-α/β-D-apio-L-furanose (4.36): A solution of **4.31** (750 mg, 2.84 mmol) in 80% aq. acetic acid (10 mL) was stirred at 80 °C for 8h. The reaction mixture was evaporated to give the crude intermediate as a syrup. This syrup was dissolved in pyridine (15 mL) and treated with DMAP (50 mg) and acetic anhydride (2.0 mL, 21.2 mmol). The solution was stirred at room temperature for 4h. The solvent was removed under vacuum and the resulting residue was purified by silica-gel column chromatography (20% EtOAc-hexanes) to yield **4.36** (500 mg, 57%) as a colorless oil (α:β anomeric ratio 1:4). Major isomer ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.94 (s, 3H, Ac) , 1.97 (s, 3H, Ac), 2.83 - 2.96 (m, 1H, 3-H), 3.40 (dd, *J* = 9.08, 7.32 Hz, 1H, 5-H), 3.55 (dd, *J* = 9.08, 7.62 Hz, 1H, 5-H), 3.77 (t, *J* = 8.79 Hz, 1H, 4-H), 4.17 (t, *J* = 8.35 Hz, 1H, 4-H), 4.35 - 4.48 (m, 2H, C*H*$_2$Ph), 5.20 (d, *J* = 4.98 Hz,

1H, 2H), 6.02 (s, 1H, 1-H), 7.18 - 7.32 (m, 5H, C*H*$_2$Ph). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.54 (*C*H$_3$CO), 21.01 (*C*H$_3$CO), 40.11 (3-C), 66.10 (5-C), 70.93 (4-C), 73.27 (*C*H$_2$Ph), 76.07 (2-C), 99.67 (1-C), 127.57 (C$_o$ *Ph*), 127.71 (C$_p$ *Ph*), 128.37 (C$_m$ *Ph*), 137.76 (C$_{ipso}$ *Ph*), 169.36 (CH$_3$*C*O), 169.71 (CH$_3$*C*O). ESI-HRMS $(M+Na)^+$ calcd: 331.1158; found: 331.1152.

1-*O*-Methyl-2-*O*-acetyl-3-deoxy-5-(*O*-benzyl)-β-D-apio-L-furanose (4.37): A solution of **4.31** (2.26 g, 8.55 mmol) and *p*-TsOH (700 mg, 4.06 mmol) in MeOH (60 mL) was stirred at room temperature for 16h, neutralized with TEA and evaporated. The residue was partitioned between EtOAc and water, organic layer separated, dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography. The intermediate was dissolved in pyridine (15 mL), acetic anhydride (2.4 mL, 25.2 mmol) and DMAP (200 mg, 1.68 mmol) were added at 0 °C and the reaction mixture was stirred at room temperature for 4h. The reaction mixture was evaporated, and partitioned between EtOAc and 10% aq. $KHSO_4$. The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by silica gel column chromatography (15% EtOAc-hexanes) to give **4.37** (1.85 g, 77%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.00 (s, 3H, 2-OAc), 2.88-3.02 (m, 1H, 3-H), 3.34 (s, 3H, 1-OMe), 3.46 (dd, *J* = 9.08, 7.32 Hz, 1H, 5-H), 3.62 (dd, *J* = 9.23, 7.18 Hz, 1H, 5-H), 3.78 (t, *J* = 8.35 Hz, 1H, 4-H), 4.14 (t, *J* = 8.49 Hz, 1H, 4-H), 4.46 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.52 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.83 (s, 1H, 1-H), 5.16 (d, *J* = 5.27 Hz, 1H, 2-H), 7.27 - 7.39 (m, 5H, CH$_2$*Ph*).

1,2-*O*-Isopropylidene-β-D-apio-L-furanose (4.8): Compound **4.7** (5.0g, 21.72 mmol) was dissolved in 50 mL of 2:1 acetic acid-water mixture and stirred at room temperature for 3 days. Solvents were evaporated in vacuo and silica gel column chromatography of the residue (50% EtOAc-hexanes) afforded the title compound **4.8** as white solid (3.4 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.33 (s, 3H, C(CH$_3$)$_2$), 1.51 (s, 3H, C(CH$_3$)$_2$), 2.12 (t, *J* = 5.93 Hz, 1H, 5-OH), 2.69 (s, 1H, 3-OH), 3.71 (dd, *J* = 6.28, 11.16 Hz, 1H, 4-H), 3.80 (d, *J* = 9.77 Hz, 1H, 5-H), 3.94 (d, *J* = 9.44 Hz, 1H,

5-H), 3.96 (dd, *J* = 5.41, 7.50 Hz, 1H, 4-H), 4.38 (d, *J* = 3.84 Hz, 1H, 2-H), 5.99 (d, *J* = 3.66 Hz, 1H, 1-H).

1,2-*O*-Isopropylidene-5-(*O*-benzyl)-β-D-apio-L-furanose (4.29): Compound **4.8** (3.1 g, 16.3 mmol) and dibutyltin oxide (6.7 g, 26.9 mmol) was dissolved in toluene (120 mL) refluxed at 140 °C for 2h. The reaction mixture was allowed to attain 100 °C then added tetrabutylammonium bromide (2.63 g, 8.15 mmol) and benzyl bromide (3.0 mL, 25.26 mmol). The reaction mixture was stirred at this temperature for 18h. Solvent was evaporated under reduced pressure and the residue purified by silica gel column chromatography (30% EtOAc-hexanes) to afford **4.29** (4.3 g, 94%) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32 (s, 3H, $C(CH_3)_2$b), 1.48 (s, 3H, $C(CH_3)_2$a), 2.76 (d, *J* = 0.88 Hz, 1H, 3-OH), 3.54 (d, *J* = 9.67 Hz, 1H, 5-H), 3.80 (d, *J* = 9.67 Hz, 1H, 5-H), 3.82 (dd, *J* = 9.37, 0.88 Hz, 1H, 4-Ha), 3.88 (dd, *J* = 9.37 Hz, 1H, 4-Hb), 4.35 (dd, *J* = 3.51, 0.88 Hz, 1H, 2-H), 4.57 (d, *J* = 12.01 Hz, 1H, PhCH_2), 4.64 (d, *J* = 12.01 Hz, 1H, PhCH_2), 5.98 (d, *J* = 3.51 Hz, 1H, 1-H), 7.27 - 7.42 (m, 5H, *Ph*CH_2).

1,2,3-Tri-(*O*-acetyl)-5-(*O*-benzyl)-β-D-apio-L-furanose (4.38): Following the similar procedure described for the synthesis of **4.35**, 2.5g (8.92 mmol) of **4.29** rendered pure product **4.38** (2.45 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.96 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 3.89 (d, *J* = 10.54 Hz, 1H, 4-H), 4.05 (d, *J* = 10.25 Hz, 1H, 4-H), 4.22 (d, *J* = 10.25 Hz, 1H, 5-H), 4.34 (d, *J* = 10.25 Hz, 1H, 5-H), 4.50 - 4.62 (m, 2H, PhCH_2), 5.49 (d, *J* = 1.17 Hz, 1H, 2-H), 6.08 (d, *J* = 1.17 Hz, 1H, 1-H), 7.26 - 7.40 (m, 5H, *Ph*CH_2).

General condition for Vorbrüggen coupling reaction: All operations were carried out under an argon protected atmosphere.

Silylation of nucleobases: The nucleobase (N^6-benzoyl protected in case of adenine) (2 eq.) was suspended in hexamethyldisilazane (50 eq.) containing trimethylsilyl chloride (0.7 eq.) and pyridine (10 eq.). The mixture was heated at reflux overnight. After cooling, the solvent was evaporated and dried under high vacuum.

Coupling at ambient condition (A): To the silylated nucleobase was added compound **4.35**/**36**/**37**/**38**/**72** (1 eq.) dissolved in dry 1,2-dichloroethane (7 mL/mmol), and trimethylsilyl triflate or anhydrous $SnCl_4$ (2.5 eq.) was added dropwise at room temperature. The clear solution was stirred for stipulated time at same temperature.

Coupling under microwave condition (B): To the silylated nucleobase was added compound **4.35**/**36**/**37**/**38**/**72** (1 eq.) dissolved in dry acetonitrile (7 mL/mmol), followed by the addition of trimethylsilyl triflate (0.2 eq.) at room temperature. The clear solution was irradiated to microwave (continuous power-300W, preheating 0 °C→150 °C in 3 min, at 150 ± 3 °C for 5 min).

Workup procedure: The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated. Purification of the residue by silica-gel flash column chromatography (MeOH-CH_2Cl_2) afforded the pure coupled product as white foam.

1′-(Thymin-1-yl)-2′-*O*-acetyl-3′-deoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.39): Using condition A, compound **4.36** (320 mg, 1.04 mmol) gave compound **4.39** (420 mg) in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.84 (d, *J* = 0.88 Hz, 3H, 5-CH_3), 1.98 (s, 3H, 2′-OAc), 2.67 - 2.85 (m, 1H, 3′-H), 3.39 (dd, *J* = 9.08, 7.62 Hz, 1H, 5′-H), 3.57 (dd, *J* = 9.23, 6.00 Hz, 1H, 5′-H), 3.89 (t, *J* = 8.93 Hz, 1H, 4′-H), 4.36 (t, *J* = 8.05 Hz, 1H, 4′-H), 4.43 (s, 2H, C*H*$_2$Ph), 5.39 (dd, *J* = 6.15, 2.34 Hz, 1H, 2′-H), 5.74 (d, *J* = 2.34 Hz, 1H, 1′-H), 6.89 - 7.02 (d, *J* = 0.88 Hz, 1H, 6-H), 7.21 - 7.38 (m, 5H, CH_2*Ph*), 8.85 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.57 (5-CH_3), 20.57 (2′-OCO*C*H_3), 41.01 (3′-C), 66.31 (5′-C), 71.87 (4′- C), 73.52 (*C*H_2Ph), 91.20 (1′-C), 110.99 (5-C), 127.71 (CH_2*Ph*), 127.86 (CH_2*Ph*), 128.45 (CH_2*Ph*), 135.13 (6-C), 137.58 (CH_2*Ph*), 149.97 (2-C), 163.68 (4- C), 169.65 (2′-O*C*OCH$_3$). ESI-HRMS $(M+H)^+$ calcd: 375.1556; found: 375.1556.

1′-(*N*^{6}-Benzoyladenin-9-yl)-2′-*O*-acetyl-3′-deoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.40): Using condition B, compound **4.37** (1.0 g, 3.56 mmol) gave compound **4.40** (1.0 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.00 (s, 3H, 2′-OAc), 3.11 - 3.25 (m,

1H, 3′-H), 3.49 (dd, *J* = 9.23, 7.18 Hz, 1H, 5′-H), 3.63 (dd, *J* = 9.37, 6.44 Hz, 1H, 5′-H), 4.00 (t, *J* = 8.49 Hz, 1H, 4′-H), 4.45 (s, 2H, PhC*H*$_2$), 4.50 (t, *J* = 8.05 Hz, 1H, 4′-H), 5.79 (dd, *J* = 5.86, 2.05 Hz, 1H, 2′-H), 6.04 (d, *J* = 2.34 Hz, 1H, 1′-H), 7.21 - 7.32 (m, 5H, CH$_2$*Ph*), 7.37 - 7.47 (m, 2H, H$_m$ Bz), 7.47 - 7.56 (m, 1H, H$_p$ Bz), 7.90 - 7.97 (m, 2H, H$_o$ Bz), 7.98 (s, 1H, 8-H), 8.72 (s, 1H, 2-H), 9.11 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.58 (2′-OCO*C*H$_3$), 41.01 (3′-C), 66.15 (5′-C), 72.12 (4′-C), 73.44 (*C*H$_2$Ph), 76.96 (2′-C), 90.24 (1′- C), 123.57 (5-C), 127.66 (C$_o$, C$_p$ Bn), 127.82 (C$_o$ Bz), 128.44 (C$_m$ Bn), 128.76 (C$_m$ Bz), 132.68 (C$_p$ Bz), 133.58 (C$_{ipso}$ Bz), 137.64 (C$_{ipso}$ Bn), 141.33 (8-C), 149.54 (6-C), 151.19 (4-C), 152.79 (2-C), 164.59 (N^{6}Bz-*C*O), 169.90 (2′-O*C*OCH$_3$). ESI-HRMS $(M+H)^+$ calcd: 488.1934; found: 488.1937.

Spectral data for compound **1′-(*N*^{6}-Benzoyladenin-1-yl)-3′-deoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.41):** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.45 - 2.61 (m, 1H, 3′-H), 3.68 (dd, *J* = 9.4, 6.4 Hz, 1H, 5′-H), 3.76 (dd, *J* = 9.5, 6.3 Hz, 1H, 5′-H), 4.22 (t, *J* = 8.79 Hz, 1H, 4′-H), 4.41 (t, *J* = 8.20 Hz, 1H, 4′-H), 4.45 - 4.52 (m, 2H, C*H*$_2$Ph), 4.62 (d, *J* = 4.98 Hz, 1H, 2′-H), 6.56 (s, 1H, 1′-H), 7.19 - 7.28 (m, 5H, CH$_2$*Ph*), 7.29 - 7.37 (m, 2H, H$_m$ Bz), 7.39 - 7.47 (m, 1H, H$_p$ Bz), 7.97 (s, 1H, 8-H), 8.16 - 8.22 (m, 2H, H$_o$ Bz), 8.40 (s, 1H, 2-H), 12.45 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 41.20 (3′-C), 65.93 (5′-C), 72.06 (4′-C), 73.56 (*C*H$_2$Ph), 77.60 (2′-C), 96.62 (1′-C), 114.65 (5-C), 127.90 (C$_o$ Bn), 128.17 (C$_p$ Bn), 128.48 (C$_m$ Bz), 128.75 (C$_m$ Bn), 129.94 (C$_o$ Bz), 132.41 (C$_p$ Bz), 137.51 (C$_{ipso}$ Bz), 137.80 (C$_{ipso}$ Bn), 141.99 (8-C), 142.16 (2-C), 148.83 (6-C), 157.95 (4-C), 175.46 (Bz *C*O). ESI-HRMS $(M+H)^+$ calcd: 446.1828; found: 446.1839.

1′-(Thymin-1-yl)-3′-deoxy-β-D-apio-L-furanose (4.43): Spectral data for the compound mixture **4.43** (minor) **+ 4.52a:** ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.77 (d, *J* = 0.88 Hz, 1.06H, minor 5-CH$_3$), 1.80 (d, *J* = 1.17 Hz, 2.89H, 5-CH$_3$, major), 2.22 - 2.36 (m, 1H, 3′-H, major), 2.52-2.60 (m, 0.29H, 3′-H, minor) 3.40 - 3.52 (m, 1.43H, 5′-H, major & minor), 3.62 - 3.72 (m, 1.43H, 5′-H, major & minor), 3.73 - 3.81 (m, 1.11H, 4′-H, major), 3.81-3.87 (m, 0.31H, 4′-H, minor), 9.95-4.02 (t, *J* =7.91 Hz, 0.39H, 4′-H, minor), 4.11-4.16 (m, 0.36H, 2′-H, minor), 4.19 (td, *J* = 5.13, 2.05 Hz, 1.05H, 2′-H, major), 4.33 (t, *J* = 7.91 Hz, 1H, 4′-H, major), 4.51 (t, *J* = 5.13 Hz,

1.34H, 5′-OH, major & minor), 5.29 (d, *J* = 4.69 Hz, 0.37H, 2′-OH, minor), 5.51 (d, *J* = 4.98 Hz, 1.02H, 2′-OH, major), 5.61 (d, *J* = 2.05 Hz, 1H, 1′-H, major), 5.88 (d, *J* = 3.22 Hz, 0.36H, 1′-H, minor), 7.30 (d, *J* = 1.17 Hz, 0.36H, 6-H, minor), 7.38 (d, *J* = 1.17 Hz, 1.02H, 6-H, major), 11.21 (s, 0.39H, NH, minor), 11.27 (s, 1.01H, major). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.11 (5-CH_3, major), 12.24 (5-CH_3, minor), 43.57 (3′-C, major), 46.06 (3′-C, minor), 57.63 (5′-C, major), 57.74 (5′-C, minor), 69.31 (2′-C, minor), 69.84 (4′-C, minor), 71.16 (4′-C, major), 74.21 (2′-C, major), 87.92 (1′-C, minor), 92.24 (1′-C, major), 106.84 (5-C, minor), 108.92 (5-C, major), 135.74 (6-C, major), 137.99 (6-C, minor), 150.32 (2-C, major), 150.39 (2-C, minor), 163.93 (4-C, major), 164.07 (4-C, minor).

1′-(Thymin-1-yl)-2′,3′-di(*O*-acetyl)-5′-*O*-benzyl-α-D-apiose-L-furanose (4.44): Using condition – A, compound **4.38** (100 mg, 0.27 mmol) gave compound **4.44** (100 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.95 (d, *J* = 1.17 Hz, 3H, 5-C*H*$_3$), 2.04 (s, 3H, Ac), 2.08 (s, 3H, Ac), 3.88 (s, 2H, 5′-H), 4.20 (d, *J* = 10.54 Hz, 1H, 4′-H), 4.55 (s, 2H, C*H*$_2$Ph), 4.56 (d, *J* = 10.54 Hz, 1H, 4′-H), 5.63 (d, *J* = 4.98 Hz, 1H, 2′-H), 5.96 (d, *J* = 4.98 Hz, 1H, 1′-H), 7.28 (d, *J* = 1.17 Hz , 1H, 6-H), 7.30 - 7.41 (m, 5H, CH$_2$*Ph*), 8.52 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.69 (5-*C*H$_3$), 20.51 (Ac-*C*H$_3$), 21.58 (Ac-*C*H$_3$), 66.68 (5′-C), 73.20 (4′-C), 73.80 (*C*H$_2$Ph), 78.06 (2′-C), 86.24 (3′-C), 88.23 (1′-C), 111.44 (5-C), 127.81 (C$_o$ Bn), 128.04 (C$_p$ Bn), 128.54 (C$_m$ Bn), 135.03 (6-C), 137.27 (C$_{ipso}$ Bn),150.19 (2-C), 163.30 (4-C), 169.13 (Ac-*C*O), 169.94 (Ac-*C*O). ESI-HRMS $(M+H)^+$ calcd: 433.1611; found: 433.1924.

1′-(*N*^{6}-Benzoyladenin-9-yl)-2′,3′-di(*O*-acetyl)-5′-*O*-benzyl-α-D-apio-L-furanose (4.45): Using condition- B, compound **4.38** (220 mg, 0.6 mmol) gave compound **4.45** (130 mg, 40%) and **4.46** (20 mg, 6%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.04 (s, 3H, Ac), 2.07 (s, 3H, Ac), 3.94 - 4.04 (2d, *J* = 9.96 Hz, 2H, 5′-H), 4.37 (d, *J* = 10.54 Hz, 1H, 4′-H), 4.59 (s, 2H, C*H*$_2$Ph), 4.71 (d, *J* = 10.54 Hz, 1H, 4′-H), 6.13 (d, *J* = 4.39 Hz, 1H, 2′-H), 6.17 (d, *J* = 4.10 Hz, 1H, 1′-H), 7.31 - 7.39 (m, 5H, CH$_2$*Ph*), 7.51 - 7.56 (m, 2H, H$_m$ Bz), 7.58 - 7.62 (m, 1H, H$_p$ Bz), 8.00 - 8.05 (m, 2H, H$_o$ Bz) 8.23 (s, 1H, 8-H), 8.82 (s, 1H, 2-H), 9.01 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.48 (Ac-*C*H$_3$), 21.53 (Ac-*C*H$_3$), 66.32 (5′-C) 73.79 (*C*H$_2$Ph & 4′-C), 78.65 (2′-C), 86.43 (3′-C),

87.93 (1′-C), 123.14 (5-C), 127.81 (C_o Bn) 127.83 (C_o Bz), 128.05 (C*p* Bn), 128.54 (C_m Bn), 128.85 (C_m Bz), 132.77 (C_m Bz), 133.59 (C_{ipso} Bn), 137.28 (C_{ipso} Bz), 141.05 (8-C), 149.51 (4-C), 151.79 (6-C), 152.94 (2-C), 164.47 (N^6*C*OPh), 168.89 (*C*OCH_3), 169.94 (*C*OCH_3). ESI-HRMS $(M+H)^+$ calcd: 546.1989; found: 546.2000. Spectral data for compound **1′-(*N*^{6}-Benzoyladenin-9-yl)-2′-(*O*-trimethylsilyl)-3′-(*O*-acetyl)-5′-*O*-benzyl-α-D-apio-L-furanose (4.46):** ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.14 (s, 9H, 2′-OSi$(CH_3)_3$) 1.89 (s, 3H, 3′-Ac) 3.96 (d, *J* = 9.96 Hz, 1H, 5′-H) 4.05 (d, *J* = 9.67 Hz, 1H, 5′-H) 4.34 (d, *J* = 10.54 Hz, 1H, 4′-H) 4.49 (d, *J* = 11.72 Hz, 1H, C*H*$_2$Ph) 4.57 (d, *J* = 12.01 Hz, 1H, C*H*$_2$Ph) 4.71 (d, *J* = 10.54 Hz, 1H, 4′-H) 5.05 (d, *J* = 2.64 Hz, 1H, 2′-H) 6.04 (d, *J* = 2.64 Hz, 1H, 1′-H) 7.27 - 7.39 (m, 5H, CH_2*Ph*) 7.50 - 7.56 (m, 2H, H_m Bz) 7.57 - 7.62 (m, 1H, H_p Bz) 8.00 - 8.06 (m, 2H, H*o* Bz) 8.15 (s, 1H, 8-H) 8.81 (s, 1H, 2-H) 9.08 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -0.12 ($SiCH_3$), 21.55 (Ac-*C*H_3), 65.96 (5′-C), 73.70 (*C*H_2Ph), 74.39 (4′-C), 78.99 (2′-C), 88.13 (3′-C), 91.74 (1′-C), 123.44 (5-C), 127.69 (C_p Bn), 127.85 (C_o Bz), 127.86 (C_o Bn), 128.43 (C_m Bn), 128.86 (C_m Bz), 132.77 (C_p Bz), 133.65 (C_{ipso} Bn), 137.61 (C_{ipso} Bz), 141.27 (8-C), 149.39 (4-C), 151.33 (6-C), 152.67 (2-C), 164.56 (N^6Bz-*C*O), 169.97 (Ac-*C*O). ESI-HRMS $(M+H)^+$ calcd: 576.2278; found: 576.2291.

1′-(Thymin-1-yl)-3′-deoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.47): Acetyl protected compound **4.39** (400 mg, 1.07 mmol) was dissolved in 7N ammonia in MeOH (15 mL). The mixture was stirred at room temoerature until completion (for about 3-5h) as indicated by TLC. Solvent was evaporated and the residue was purified by flash column chromatography using 0.5-1 % MeOH-CH_2Cl_2 to afford the title compound **4.47** (341 mg, 96%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.83 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.27 - 2.49 (m, 1H, 3′-H), 3.57 (dd, *J* = 9.23, 7.76 Hz, 1H, 5′-H), 3.78 (dd, *J* = 9.23, 6.00 Hz, 1H, 5′-H), 4.03 (dd, *J* = 10.54, 8.49 Hz, 1H, 4′-H), 4.29 - 4.38 (m, 2H, 4′-H & 2′-H), 4.45 (s, 2H, C*H*$_2$Ph), 5.02 (br s, 1H, 2′-OH), 5.67 (s, 1H, 1′-H), 7.11 (d, *J* = 1.17 Hz, 1H, 6-H), 7.19 - 7.30 (m, 5H, CH_2*Ph*), 10.44 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.60 (5-CH_3), 41.34 (3′- C), 66.61 (5′-C), 72.88 (4′-C), 73.55 (*C*H_2Ph), 75.79 (2′-C), 94.33 (1′-C), 110.49 (5-C),

127.72 (CH_2*Ph*), 127.74 (CH_2*Ph*), 128.39 (CH_2*Ph*), 134.67 (6- C), 137.88 (CH_2*Ph*), 150.61 (2-C), 164.47 (4-C). ESI-HRMS $(M+H)^+$ calcd: 333.1450; found: 333.1458.

1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.48): Compound **4.40** (1.0 g, 2.05 mmol) was dissolved in 7N ammonia in MeOH (30 mL). The mixture was stirred at room temperature for 48h. Solvent was evaporated and the residue was purified by flash column chromatography using 2% MeOH-CH_2Cl_2 to afford the title compound **4.48** (650 mg, 75%) as a white foam [Procedure to remove acetamide residue if any: Suspend the product in water and then collect it by filtration]. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.75 - 2.89 (m, 1H, 3′-H), 3.53 (t, *J* = 8.79 Hz, 1H, 5′-H), 3.73 (dd, *J* = 9.37, 5.86 Hz, 1H, 5′-H), 3.86 (t, *J* = 8.20 Hz, 1H, 4′-H), 4.40 (t, *J* = 7.76 Hz, 1H, 4′-H), 4.45 - 4.56 (m, 2H, Bn H), 4.63 (td, *J* = 5.27, 2.05 Hz, 1H, 2′-H), 5.76 (d, *J* = 4.69 Hz, 1H, 2′-OH), 5.90 (d, *J* = 2.34 Hz, 1H, 1′-H), 7.26 (br s, 2H, NH), 7.27 - 7.39 (m, 5H, CH_2*Ph*), 8.15 (s, 1H, 2-H), 8.23 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.70 (3′-C), 66.77 (5′-C), 71.09 (4′-C), 72.25 (Bn C), 74.38 (2′-C), 91.11 (1′-C), 119.16 (5-C), 127.38 (C_o Bn), 127.49 (C_p Bn), 128.21 (C_m Bn), 138.46 (C_{ipso} Bn), 138.98 (8-C), 148.80 (4-C), 152.52 (2-C), 156.00 (6-C). ESI-HRMS $(M+H)^+$ calcd: 342.1566; found: 342.1565.

1′-(Thymin-1-yl)-5′-*O*-benzyl-α-D-apio-L-furanose (4.49): Following a similar procedure described for compound **4.47**, compound **4.44** (100 mg, 0.23 mmol) gave compound **4.49** (81 mg, 86 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.81 (d, *J* = 0.88 Hz, 3H, 5-CH_3), 3.60 (d, *J* = 9.67 Hz, 1H, 4′-H), 3.85 (d, *J* = 9.67 Hz, 1H, 4′-H), 3.90 (d, *J* = 1.17 Hz, 1H, 3′-OH), 4.06 (dd, *J* = 9.37, 1.47 Hz, 1H, 5′-H), 4.17 (d, *J* = 9.37 Hz, 1H, 5′-H), 4.41 (d, *J* = 3.51 Hz, 1H, 2′-H), 4.50 - 4.69 (app-q, *J* = 12.01 Hz, 2H, C*H*$_2$Ph), 5.25 (d, *J* = 3.51 Hz, 1H, 2′-OH), 5.72 (s, 1H, 1′-H), 7.23 - 7.37 (m, 5H, CH_2*Ph*), 7.52 (d, *J* = 1.17 Hz, 1H, 6-H), 10.81 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.41 (5-*C*H_3), 69.57 (4′-C), 73.72 (*C*H_2Ph), 77.01 (5′-C), 79.96 (2′-C), 80.54 (3′-C), 94.36 (1′-C), 108.63 (5-C), 127.77 (C_o Bn), 127.84 (C_p Bn), 128.42 (C_m Bn), 137.55 (C_{ipso} Bn), 137.62 (6-C) 151.28 (2-C), 164.83 (4-C). ESI-HRMS $(M+H)^+$ calcd: 349.1400; found: 349.1384.

1′-(Adenin-9-yl)-5′-*O*-benzyl-α-D-apio-L-furanose (4.50): Following a similar procedure described for compound **4.48**, compound **4.45** (120 mg, 0.22 mmol) gave compound **4.50** (73 mg, 93%) as a white foam. The same procedure was employed to convert **4.46** to **4.50**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.57 - 3.70 (2d, *J* = 9.67 Hz, 2H, 5′-H), 4.00 (d, *J* = 8.79 Hz, 1H, 4′-H), 4.11 (d, *J* = 9.08 Hz, 1 H4′-H), 4.39 (dd, *J* = 5.27, 2.93 Hz, 1H, 2′-H) 4.51 - 4.64 (2d, *J* = 12.30 Hz, 2H, C*H*$_2$Ph), 5.59 (s, 1H, 3′-OH), 5.90 (d, *J* = 2.93 Hz, 1H, 1′-H), 5.97 (d, *J* = 5.56 Hz, 1H, 2′-OH), 7.27 (s, 2H, NH), 7.28 - 7.43 (m, 5H, CH$_2$*Ph*) 8.15 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 71.12 (5′-C), 72.70 (*C*H$_2$Ph), 75.55 (4′-C), 79.84 (3′-C), 80.28 (2′-C), 90.68 (1′-C), 118.78 (5- C), 127.34 (C$_p$ Bn), 127.44 (C$_o$ Bn), 128.19 (C$_m$ Bn), 138.50 (C$_{ipso}$ Bn) 139.68 (8-C) 149.03 (4-C) 152.46 (2-C) 155.98 (6-C). ESI-HRMS (M+H)$^+$ calcd: 358.1515; found: 358.1512.

1′-(Thymin-1-yl)-3′-deoxy-α-D-apio-L-furanose (4.52a): Compound **4.47** (300 mg, 0.9 mmol) was dissolved in MeOH (10 mL), to this was added Pd-C (300 mg, 10% Pd, wet ~50%). A stream of hydrogen gas was bubbled through the reaction mixture with vigorous stirring for about 1h and the mixture was then stirred under hydrogen atmosphere overnight at room temperature. The catalyst was filtered off, the filtrate was concentrated and purified by silica-gel flash column chromatography eluting with 6-8% MeOH-CH_2Cl_2 to afford compound **4.52a** (190 mg, 86%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.79 (d, *J* = 1.17 Hz, 3H, 5-CH$_3$), 2.22 - 2.36 (m, 1H, 3′-H), 3.46 (ddd, *J* = 10.76, 7.69, 5.27 Hz, 1H, 5′-H), 3.62 - 3.71 (m, 1H, 5′-H), 3.76 (t, *J* = 8.64 Hz, 1H, 4′-H), 4.18 (td, *J* = 4.98, 2.05 Hz, 1H, 2′-H), 4.33 (t, *J* = 7.76 Hz, 1H, 4′-H), 4.51 (t, *J* = 5.13 Hz, 1H, 5′-OH), 5.51 (d, *J* = 4.69 Hz, 1H, 2′-OH), 5.61 (d, *J* = 2.05 Hz, 1H, 1′-H), 7.38 (d, *J* = 1.17 Hz, 1H, 6-H), 11.27 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.09 (5-CH$_3$), 43.55 (3′-C), 57.61 (5′-C), 71.14 (4′-C), 74.19 (2′-C), 92.22 (1′-C), 108.90 (5-C), 135.73 (6-C), 150.30 (2-C), 163.92 (4-C). ESI-HRMS (M+H)$^+$ calcd: 243.0981; found: 243.0990.

1′-(Adenin-9-yl)-3′-deoxy-α-D-apio-L-furanose (4.52b): Compound **4.48** (450 mg, 1.32 mmol) was dissolved in 1:1 v/v mixture of MeOH-formic acid (40 mL), to this was added Pd(OH)$_2$-C (300 mg, 10% Pd, wet ~50%) and stirred at 55 °C for 5-8h . The

catalyst was filtered off and the filtrate was concentrated. The residue contained compound **4.52b** and **4.51** as mixture. The residue was dissolved in 7N NH_3-MeOH and stirred at room temperature for 3h. The volatiles were evaporated and the residue purified by silica-gel flash column chromatography eluting with 10-12% MeOH-CH_2Cl_2 to afford compound **4.52b** (265 mg, 80%) as a white solid. Spectral data for **1′-(adenin-9-yl)-3′-deoxy-5′-O-formyl-α-D-apio-L-furanose (4.51):** ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.82 - 3.00 (m, 1H, 3′-H), 3.86 (t, *J* = 8.20 Hz, 1H, 4′-H), 4.21 (dd, *J* = 10.98, 7.76 Hz, 1H, 5′-H), 4.32 - 4.46 (m, 2H, 4′ & 5′-H's), 4.70 (br s, 1H, 2′-H), 5.93 (d, *J* = 2.05 Hz, 1H, 1′-H), 7.28 (s, 2H, NH), 8.15 (s, 1H, 2-H), 8.24 (s, 1H, 5′-OCOH), 8.25 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.34 (3′-C), 61.27 (5′-C), 71.06 (4′-C), 74.73 (2′-C), 91.80 (1′-C), 119.73 (5-C), 139.85 (8-C), 149.44 (4-C), 153.29 (2-C), 156.51 (6-C), 162.81 (5′-O*C*OH). ESI-HRMS $(M+H)^+$ calcd: 280.1046; found: 280.1046. Spectral data for **1′-(adenin-9-yl)-3′-deoxy-α-D-apio-L-furanose (4.52b):** ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.53 - 2.66 (m, 1H, 3′-H), 3.52 (t, *J* = 8.79 Hz, 1H, 5′-H), 3.67 - 3.77 (m, 1H, 5′-H), 3.86 (t, *J* = 8.20 Hz, 1H, 4′-H), 4.35 (t, *J* = 7.76 Hz, 1H, 4′-H), 4.54 (br s, 1H, 5′-OH), 4.63 (br s, 1H, 2′-H), 5.64 (d, *J* = 4.69 Hz, 1H, 2′-OH), 5.89 (d, *J* = 2.34 Hz, 1H, 1′-H), 7.25 (br s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.22 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.10 (3′-C), 57.64 (5′-C), 70.84 (4′-C), 74.39 (2′-C), 91.11 (1′-C), 119.19 (5- C), 138.93 (8-C), 148.80 (4-C), 152.51 (2-C), 156.00 (6-C). ESI-HRMS $(M+H)^+$ calcd: 252.1097; found: 252.1090.

1′-(Thymin-1-yl)-α-D-apio-L-furanose (4.53a): Following a similar procedure described for compound **4.52a**, compound **4.49** (210 mg, 0.60 mmol) gave compound **4.53a** (110 mg, 71%) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.77 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 3.54 (s, 2H, 5′-H), 3.88 (d, *J* = 9.08 Hz, 1H, 4′-H), 3.93 (br s, 1H, 2′-H), 3.98 (d, *J* = 9.08 Hz, 1 H), 4.57 (br s, 1H, 3′-OH), 5.04 (s, 1H, 5′-OH), 5.67 (d, *J* = 2.64 Hz, 1H, 1′-H), 5.72 (d, *J* = 4.69 Hz, 1H, 2′-OH), 7.62 (d, *J* = 1.17 Hz, 1H, 6-H), 11.25 (br s, 1H, NH).^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.98 (5-*C*H_3), 62.95 (5′-C), 76.52 (4′-C), 80.51 (2′-C), 81.00 (3′-C), 92.95 (1′-C), 108.70 (5-C),

137.90 (6-C), 151.19 (2-C), 164.62 (4-C). ESI-HRMS $(M+H)^+$ calcd: 259.0930; found: 259.0927.

1′-(Adenin-9-yl)-α-D-apio-L-furanose (4.53b): Compound **4.50** (20 mg, 0.056 mmol) was dissolved in 9:1 v/v mixture MeOH-formic acid (2 mL), to this was added $Pd(OH)_2$-C (20 mg, 10% Pd, wet ~50%) and stirred at 55 °C for 5-8h . The catalyst was filtered off, the filtrate was concentrated and the residue was purified by silica-gel flash column chromatography eluting with 10-14% MeOH-CH_2Cl_2 to afford compound **4.53b** (12 mg, 80%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.62 (d, *J* = 5.56 Hz, 2H, 5′-H), 3.98 (d, *J* = 9.08 Hz, 1H, 4′-H), 4.04 (d, *J* = 9.08 Hz, 1H, 4′-H), 4.38 (br s, 1H, 2′-H), 4.64 (t, *J* = 5.71 Hz, 1H, 5′-OH), 5.36 (s, 1H, 3′-OH), 5.85 (d, *J* = 4.69 Hz, 1H, 2′-OH), 5.90 (d, *J* = 2.93 Hz, 1H, 1′-H), 7.26 (s, 2H, NH), 8.15 (s, 1H, 2-H), 8.31 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 62.04 (5′-C), 75.32 (4′-C), 80.13 (2′-C), 80.33 (3′-C), 90.60 (1′-C), 118.70 (5-C), 139.64 (8-C), 148.95 (4-C), 152.34 (2-C), 155.88 (6-C). ESI-HRMS $(M+H)^+$ calcd: 268.1046; found: 268.1036.

1′-(Thymin-1-yl)-3′-deoxy-5′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (4.54): Compound **4.52a** (150 mg, 0.62 mmol) was dissolved in DMF (3.5 mL), to this was added imidazole (85 mg, 1.24 mmol) followed by *tert*-butyldimethylsilylchloride (TBDMSCl) (112 mg, 0.74 mmol). The mixture was stirred at room temperature for 18h. DMF was evaporated under reduced pressure. The residue was partitioned between EtOAc and brine. Organic layer separated, dried over sodium sulphate, solvent evaporated and the residue purified by silica-gel flash column chromatography using 1-2% MeOH-CH_2Cl_2 to afford compound **4.54** (210 mg, 95%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.07 (2s, 6H, $Si(CH_3)_2$), 0.89 (S, 9H, $C(CH_3)_3$), 1.94 (d, *J* = 0.88 Hz, 3H, 5-CH_3), 2.32-2.46 (m, 1H, 3′-H), 3.84 (d, *J* = 10.25, 7.32 Hz, 1H, 5′-H), 3.97 (d, *J* = 10.25, 5.86 Hz, 1H, 5′-H), 4.10 (t, *J* = 8.48 Hz, 1H, 4′-H), 4.35 (t, *J* = 7.91 Hz, 1H, 4′-H), 4.39 (t, *J* = 4.1 Hz, 1H, 2′-H), 4.81 (d, *J* = 3.22 Hz, 1H, 2′-OH), 5.74 (s, 1H, 1′-H), 7.22 (d, *J* = 1.17 Hz, 1H, 6-H), 10.19 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.51 ($Si(CH_3)_2$), -5.47 ($Si(CH_3)_2$), 12.65 (5-CH_3), 18.24 ($C(CH_3)_3$), 25.85 ($C(CH_3)_3$), 43.46 (3′-C), 59.75 (5′-

C), 72.48 (4′-C), 76.10 (2′-C), 94.60 (1′-C), 110.54 (5-C), 134.84 (6-C), 150.66 (2-C), 164.35 (4-C). ESI-HRMS $(M+H)^+$ calcd: 357.1846; found: 357.1852.

1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (4.55): Following a similar procedure described for compound **4.54**, compound **4.52b** (260 mg, 1.03 mmol) afforded compound **4.55** (310 mg, 82%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.10 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$), 0.91 (s, 9H, $C(CH_3)_3$), 2.65 - 2.78 (m, 1H, 3′-H), 3.97 (dd, *J* = 6.00, 1.32 Hz, 2H, 5′-H), 4.21 (dd, *J* = 8.35, 7.47 Hz, 1H, 4′-H), 4.39 (dd, *J* = 8.49, 7.32 Hz, 1H, 4′-H), 4.81 (dt, *J* = 5.71, 2.71 Hz, 1H, 2′-H), 5.15 (d, *J* = 3.22 Hz, 1H, 2′-OH), 5.94 (br s, 2H, NH), 5.97 (d, *J* = 2.64 Hz, 1H, 1′-H), 7.94 (s, 1H, 8-H), 8.32 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.52 ($SiCH_3$), -5.50 ($SiCH_3$), 18.19 ($C(CH_3)_3$), 25.81 ($C(CH_3)_3$), 43.28 (3′-C), 60.02 (5′-C), 71.17 (4′-C), 77.07 (2′- C), 93.00 (1′-C), 120.31 (5-C), 138.43 (8-C), 148.96 (4-C), 152.73 (2-C), 155.51 (6-C). ESI-HRMS $(M+H)^+$ calcd: 366.1961; found: 366.1941.

1′-(Thymin-1-yl)-2′,3′-dideoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.56): To a solution of compound **2.47** (250 mg, 0.75 mmol) and DMAP (184 mg, 1.5 mmol) in acetonitrile (10 mL) was dropwise added *O*-*p*-tolyl chlorothionoformate (138µL, 0.9 mmol) at room temperature. The mixture was stirred for additional 2h, and then the volatile organics were evaporated under reduced pressure. The residue was suspended in EtOAc and washed with water and brine. The organic layer was dried over anhydrous sodium sulphate and the solvent evaporated to dryness. The residue obtained was suspended in toluene (25 mL), tributyltinhydride (0.51 mL, 1.88 mmol) was added followed by at 60-70 °C was added azoisobutyronitrile (AIBN, 250 mg, 1.5 mmol) and heated to 110-120 °C for 3h. Volatile materials were evaporated and the residue was purified by silica-gel flash column chromatography using 0.5-2% MeOH-CH_2Cl_2 to afford compound **4.56** (167 mg, 70 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.86 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.06 (ddd, *J* = 13.77, 7.91, 3.81 Hz, 1H, 2′-H), 2.16 (ddd, *J* = 13.84, 7.54, 6.44 Hz, 1H, 2′-H), 2.52 - 2.68 (m, 1H, 3′-H), 3.36 (dd, *J* = 9.08, 7.32 Hz, 1H, 5′-H), 3.45 (dd, *J* = 9.08, 5.56 Hz, 1H, 5′-H), 3.74 (dd, *J* = 8.79, 7.03 Hz, 1H, 4′-H), 4.24 (dd, *J* = 8.79, 7.32 Hz, 1H, 4′-H), 4.45 (s,

2H, C*H*$_2$Ph), 5.96 (dd, *J* = 6.44, 4.10 Hz, 1H, 1′-H), 7.07 (d, *J* = 1.17 Hz, 1H, 6-H), 7.20 - 7.33 (m, 5H, CH$_2$*Ph*), 8.56 (br s, 1H, NH). ^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 12.65 (5-CH$_3$), 35.88 (2′-C), 38.06 (3′-C), 70.83 (5′-C), 72.71 (4′-C), 73.39 (*C*H$_2$Ph), 86.96 (1′-C), 110.38 (5-C), 127.67 (CH$_2$*Ph*), 127.85 (CH$_2$*Ph*), 128.49 (CH$_2$*Ph*), 135.04 (6-C), 137.76 (CH$_2$*Ph*), 150.09 (2-C), 163.72 (4-C). ESI-HRMS (M+H)$^+$ calcd: 317.1501; found: 317.1499.

1′-(Adenin-9-yl)-2′,3′-dideoxy-5′-*O*-benzyl-α-D-apio-L-furanose (4.57): Following a similar procedure described for compound **4.56**, compound **4.48** (45 mg, 0.13 mmol) gave compound **4.57** (30 mg, 70 %) as a white foam. ^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 2.32 (ddd (dt), *J* = 13.91, 7.10 Hz, 1H, 2′-H), 2.68 (ddd, *J* = 13.55, 7.83, 2.93 Hz, 1H, 2′-H), 2.81 - 2.95 (m, 1H, 3′-H), 3.45 - 3.58 (m, 2H, 5′-H), 3.90 (dd, *J* = 8.79, 6.44 Hz, 1H, 4′-H), 4.34 (dd, *J* = 8.64, 7.47 Hz, 1H, 4′-H), 4.53 (s, 2H, C*H*$_2$Ph), 6.11 (br s, 2H, NH), 6.29 (dd, *J* = 6.88, 3.08 Hz, 1H, 1′-H), 7.27 - 7.39 (m, 5H, CH$_2$*Ph*), 7.90 (s, 1H, 8-H), 8.32 (s, 1H, 2- H). ^{13}C NMR (75 MHz, CDCl$_3$) δ ppm 35.47 (2′-C), 38.19 (3′-C) 71.02 (5′-C), 72.18 (4′-C), 73.27 (*C*H$_2$Ph), 85.90 (1′-C), 120.17 (5-C), 127.63 (CH$_2$*Ph*), 127.78 (CH$_2$*Ph*), 128.44 (CH$_2$*Ph*), 137.85 (CH$_2$*Ph*), 138.45 (8-C), 149.21 (4-C) 152.83 (2-C) 155.53 (6-C). ESI-HRMS (M+H)$^+$ calcd: 326.1617; found: 326.1611.

1′-(Thymin-1-yl)-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (4.58): Following a similar procedure described for compound **4.56**, compound **4.54** (190 mg, 0.53 mmol) gave compound **4.58** (130 mg, 72 %) as a white foam. ^{1}H NMR (300 MHz, CDCl$_3$) δ ppm 0.06 (s, 6H, Si(CH$_3$)$_2$), 0.90 (s, 9H, C(CH$_3$)$_3$), 1.94 (d, *J* = 1.26 Hz, 3H, 5-CH$_3$), 2.06 (ddd, *J* = 13.84, 8.18, 3.99 Hz, 1H, 2′-H), 2.25 (dt, *J* = 13.84, 6.92 Hz, 1H, 2′-H), 2.48 - 2.63 (m, 1H, 3′-H), 3.58 (dd, *J* = 9.96, 6.82 Hz, 1H, 5′-H), 3.66 (dd, *J* = 10.07, 5.24 Hz, 1H, 5′-H), 3.83 (dd, *J* = 8.81, 6.92 Hz, 1H, 4′-H), 4.26 (dd, *J* = 8.70, 7.24 Hz, 1H, 4′-H), 6.04 (dd, *J* = 6.61, 3.88 Hz, 1H, 1′-H), 7.16 (q, *J* = 1.26 Hz, 1H, 6-H), 8.88 (br s, 1H, NH). ^{13}C NMR (75 MHz, CDCl$_3$) δ ppm -5.51 (Si*C*H$_3$), -5.48 (Si*C*H$_3$), 12.65 (5-*C*H$_3$), 18.23 (*C*(CH$_3$)$_3$), 25.81 (C(*C*H$_3$)$_3$), 35.34 (2′-C), 40.14 (3′-C), 63.37 (5′-C), 72.17 (4′-C), 87.08 (1′-C), 110.35 (5-C), 135.10 (6-C), 150.20 (2-C), 163.90 (4-C). ESI-HRMS (M+H)$^+$ calcd: 341.1897; found: 341.1884.

1′-(Adenin-9-yl)-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (4.59): Following a similar procedure described for compound **4.56,** compound **4.55** (300 mg, 0.82 mmol) gave compound **4.59** (253 mg, 88 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.07 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 2.34 (ddd (dt), *J* = 13.69, 7.07 Hz, 1H, 2′-H), 2.62 (ddd, *J* = 13.33, 7.76, 2.93 Hz, 1H, 2′-H), 2.76 (dq, *J* = 13.51, 6.87 Hz, 1H, 3′-H), 3.60 - 3.74 (m, 2H, 5′-H), 3.92 (dd, *J* = 8.79, 6.44 Hz, 1H, 4′-H), 4.31 (dd, *J* = 8.49, 7.32 Hz, 1H, 4′-H), 5.70 (br s, 2H, NH), 6.30 (dd, *J* = 6.74, 2.93 Hz, 1H, 1′-H), 7.93 (s, 1H, 8-H), 8.36 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.45 ($Si(CH_3)$), -5.42 ($Si(CH_3)$), 18.26 ($C(CH_3)_3$), 25.84 ($C(CH_3)_3$), 34.94 (2′-C), 40.38 (3′-C), 63.54 (5′-C), 71.80 (4′-C), 86.08 (1′-C), 120.32 (5-C), 138.57 (8-C), 149.35 (4-C), 152.96 (2-C), 155.36 (6-C). ESI-HRMS $(M+H)^+$ calcd: 350.2012; found: 350.2006.

1′-(Thymin-1-yl)-2′,3′-dideoxy-α-D-apio-L-furanose (4.61a): Following the hydrogenation procedure described for compound **4.52a**, compound **4.56** (150 mg, 0.47 mmol) gave compound **4.61a** (80 mg, 63 %) as a white solid. Alternatively, the compound **4.58** (110 mg, 0.32 mmol) was dissolved in THF (2 mL) and TBAF (1M, 0.65 mL, 0.65 mmol) was added at room temperature. The reaction mixture was stirred for 3h, solvents evaporated, and the residue was subjected to silica-gel flash column chromatography (4-5% MeOH-CH_2Cl_2) to afford **4.61a** (65 mg, 89%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, *J* = 0.88 Hz, 3H, 5-CH_3), 1.96 - 2.13 (m, 2H, 2′-H), 2.45-2.60 (m, 1H, 3′-H), 3.33 - 3.48 (m, 2H, 5′-H), 3.63 (dd, *J* = 8.20, 6.15 Hz, 1H, 4′-H), 4.22 (dd, *J* = 8.20, 7.03 Hz, 1H, 4′-H), 4.76 (t, *J* = 5.27 Hz, 1H, 5′-OH), 5.97 (dd, *J* = 6.44, 4.69 Hz, 1H, 1′-H), 7.43 (d, *J* = 1.17 Hz, 1H, 6-H), 11.24 (s, 1H, NH). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 12.58 (5-CH_3), 36.18 (2′-C), 41.73 (3′-C), 63.92 (5′-C), 73.31 (4′-C), 88.50 (1′-C), 111.36 (5-C), 137.85 (6-C), 152.42 (2-C), 166.70 (4-C). ESI-HRMS $(M+H)^+$ calcd: 227.1032; found: 227.1041.

1′-(Adenin-9-yl)-2′,3′-dideoxy-α-D-apio-L-furanose (4.61b): Compound **4.59** (350 mg, 1.0 mmol) was dissolved in MeOH (15 mL) in a polypropylene vessel and NH_4F (742 mg, 20 mmol) was added at room temperature. The reaction mixture was stirred at 50 °C for 48h; CH_2Cl_2 (20 mL) was added to the reaction vessel and filtered. The

filtrate was evaporated, and the residue was subjected to silica-gel flash column chromatography (10-12% MeOH-CH_2Cl_2) to afford **4.61b** (205 mg, 87%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.21 (app-q, *J* = 6.74, 13.47 Hz, 1H, 2′-H), 2.54 (ddd, *J* = 3.51, 8.20, 12.89 Hz, 1H, 2′-H), 2.76 (sep, *J* = 6.44 Hz, 1H, 3′-H), 3.44 (m, 2H, 5′-H), 3.75 (dd, *J* = 5.27, 8.20 Hz, 1H, 4′-H), 4.18 (t, *J* = 7.91 Hz, 1H, 4′-H), 4.82 (t, *J* = 4.98 Hz, 1H, 5′-OH), 6.27 (dd, *J* = 3.22, 6.74 Hz, 1H, 1′-H), 7.24 (br s, 2H, 6-NH_2's), 8.15 (s, 1H, 2-H), 8.26 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) 33.88 (2′-C), 40.44 (3′-C), 62.18 (5′-C), 70.85 (4′-C), 84.31 (1′-C), 119.15 (5-C), 139.16 (8-C), 148.93 (4-C), 152.50 (2-C), 155.99 (6-C). ESI-HRMS $(M+H)^+$ calcd: 236.1147; found: 236.1131.

1′-(Thymin-1-yl)-2′,3′-dideoxy-α-D-apio-L-furanose [phenyl-(benzoxy-L-alaninyl)] phosphate (4.62a): To a solution of **4.61a** (0.095 g, 0.42 mmol) in anhydrous THF (10 mL) was added 1.0M solution of *tert*-butyl magnesium chloride in THF (0.84 mL, 0.84 mmol) and the reaction mixture was stirred under an argon atmosphere for 30 min. After this period, a solution of **4.60** (0.30 g, 0.84 mmol) in anhydrous THF (5 mL) was added dropwise and the reaction mixture was stirred at room temperature for 17h. After this period, the solvent was removed and the residue was purified by column chromatography, gradient elution of $CHCl_3$/MeOH = 98/2 to 95/5 to give **4.62a** (0.051 g, 22%) as a white solid. ^{31}P NMR (CD_3OD, 202 MHz): δ 3.80, 3.30. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.40-7.30 (8H, m, H-6, PhO, OCH_2*Ph*), 7.23-7.18 (3H, m, PhO, OCH_2*Ph*), 6.01-5.99 (0.5H, m, H-1′), 5.98-5.96 (0.5H, m, H-1′), 5.17, 5.16 (2H, 2s, O*C*H_2Ph), 4.28-4.21 (1H, m, H-4′ of one diastereoisomer), 4.14-4.00 (3H, m, 3′-CH_2, C*H*CH_3), 3.75-3.69 (1H, m, H-4′ of one diastereoisomer), 2.80-2.74 (0.5H, m, H-3′ of one diastereoisomer), 2.73-2.66 (0.5H, m, H-3′ of one diastereoisomer), 2.22-2.07 (2H, m, H-2′), 1.90 (3H, 2s, 5-CH_3), 1.38 (1.5H, d, *J* = 7.20 Hz, CHCH_3 of one diastereoisomer), 1.36 (1.5H, d, *J* = 7.4 Hz, CHCH_3 of one diastereoisomer). ^{13}C NMR (CD_3OD, 125 MHz): δ 12.56 (5-CH_3), 20.38 (d, J_{C-P} = 7.2 Hz, CH_3), 20.44 (d, J_{C-P} = 7.2 Hz, CH_3), 35.61, 35.64 (C-2′), 39.73, 39.79 (C-3′), 51.66, 51.83 (*C*HCH_3), 68.00 (O*C*H_2Ph), 68.49 (d, J_{C-P} = 6.0 Hz, 3′-*C*H_2), 68.53 (d, J_{C-P} = 5.8 Hz, 3′-*C*H_2), 72.49, 72.54 (C-4′), 88.36, 88.38 (C-1′), 111.30, 111.33 (C-5),

121.50, 121.53, 121.57, 121.61, 126.22, 128.03, 129.33, 129.37, 129.40, 129.42, 129.65, 129.66, 130.83 (arom H), 137.32 C_{ipso} Bn), 137.74, 137.76 (C-6), 152.16, 152.19, 152.23 (C-2, C_{ipso} OPh), 166.52 (C-4), 174.75 (d, J_{C-P} = 4.6 Hz, CO), 174.96 (d, J_{C-P} = 4.6 Hz, CO). ES-MS= 566.17 ($M+Na^+$). HPLC = H_2O/AcCN from 100/0 to 0/100 in 30 min = retention time 18.24 min; H_2O/MeOH from 100/0 to 0/100 in 30 min = retention time 25.07 min.

1′-(Adenin-9-yl)-2′,3′-dideoxy-α-D-apio-L-furanose [phenyl-(benzoxy-L-alaninyl)] phosphate (4.62b): To a solution of **4.61b** (0.10 g, 0.42 mmol) in anhydrous THF (10 mL) and anhydrous pyridine (2 mL) was added a solution of 34 (0.45g, 1.26 mmol) in anhydrous THF (5 mL), followed by the addition dropwise under an argon atmosphere of anhydrous N-methylimidazole (0.10 mL, 1.26 mmol) and the reaction mixture was stirred at room temperature for 24h. After this period, a solution of **4.60** (0.30 g, 0.84 mmol) in anhydrous THF (3 mL) and anhydrous N-methylimidazole (0.07 mL, 0.84 mmol) were added and the reaction mixture was stirred at room temperature for further 24h. After this period, the solvent was removed and the residue was purified by column chromatography, gradient elution of CH_2Cl_2, then CH_2Cl_2/MeOH = 98/2 then 96/4 then 90/10 to give a white solid which was triturated with diethyl ether to give **4.62b** (0.035 g, 15%) as a white solid. ^{31}P NMR (CD_3OD, 202 MHz): δ 3.86, 3.31. ^{1}H NMR (CD_3OD, 500 MHz): δ 8.22, 8.21, 8.20, 8.17 (2H, 4s, H-2, H-8), 7.37-7.16 (10H, m, arom H), 6.31 (0.5H, dd, *J* = 7.00 Hz, 3.30 Hz, H-1′ of one diastereoisomer), 6.26 (0.5H, dd, *J* = 7.00 Hz, 3.20 Hz, H-1′ of one diastereoisomer), 5.16, 5.15 (2H, 2s, C*H*$_2$Ph), 4.29-4.22 (1H, m, H-4′), 4.18-4.02 (3H, m, C*H*CH_3, 3′-CH_2), 3.86-3.78 (1H, m, H-4′), 3.03-2.89 (1H, m, H-3′), 2.65-2.56 (1H, m, H-2′), 2.35-2.24 (1H, m, H-2′), 1.39 (1.5H, d, *J* = 7.00 Hz, CH_3 of one diastereoisomer), 1.37 (1.5H, d, *J* = 7.20 Hz, CH_3 of one diastereoisomer). ^{13}C NMR (CD_3OD, 125 MHz): δ 20.35 (d, J_{C-P} = 6.70 Hz, CH*C*H_3), 20.41 (d, J_{C-P} = 6.80 Hz, CH*C*H_3), 35.38, 35.39 (C-2′), 40.00 (d, J_{C-P} = 2.70 Hz, C-3′), 40.06 (J_{C-P} = 2.80 Hz, C-3′), 51.66, 51.83 (*C*HCH_3), 67.95, 67.97 (*C*H_2Ph), 68.63 (d, J_{C-P} = 5.70 Hz, 3′-CH_2), 68.75 (d, J_{C-P} = 5.8 Hz, 3′-CH_2), 72.13, 72.15 (C-4′), 86.98 (C-1′), 120.65, 121.48, 121.52, 121.57, 121.61, 126.18, 126.21, 129.31, 129.35, 129.37, 129.61,

129.72, 130.80 (arom H), 137.31 (C_{ipso} Bn), 140.78 (C-2), 152.19 (d, J_{C-P} = 5.50 Hz, C_{ipso} OPh), 152.25 (d, J_{C-P} = 4.70 Hz, C_{ipso} OPh), 153.67, 153.81 (C-8), 157.30 (C-8), 174.75 (d, J_{C-P} = 4.70 Hz, CO), 174.96 (d, J_{C-P} = 4.50 Hz, CO). ES- MS= 575.1640 ($M+Na^+$). HPLC = H_2O/AcCN from 100/0 to 0/100 in 30 min = retention time 17.05 min.

1′-(Adenin-9-yl)-2′,3′-dideoxy-α-D-apio-L-furanose triphosphate (4.65): Compound **4.61b** (25 mg, 0.106 mmol) and tributylammonium pyrophosphate **4.64** (117 mg, 0.212 mmol) were placed in a 50 mL and a 10 mL RB flask respectively, and dried under high vacuum for 1h. 2-chloro-4H-1,3,2-benzodioxaphosphinin-4-one **4.63** (43 mg, 0.212 mmol) was placed in a separate 10 mL flask and dried briefly (10 min) under high vacuum. Anhydrous DMF (0.25 mL) was added to each flask under argon atmosphere. Tributylamine (dried and stored over 4A molecular sieves, 0.3 mL) was added to the flask containing tributylammonium pyrophosphate (**4.64**) with stirring. The contents of this flask were added to the flask containing 2-chloro-4H-1,3,2-benzodioxaphosphinin-4-one (**4.63**) and stirring continued for 1.5h. The cyclic phosphitodiphosphate formed was added to a flask containing compound **4.61b** in DMF. After stirred for 1.5h, 3% iodine solution (9:1 pyridine-water, 2.25 mL) was added drop wise and stirred for 20 min followed by the addition of water (4 mL) and stirred for additional 1.5h. 3M NaCl solution (0.66 mL) was added to the reaction mixture. The reaction mixture was transferred to two centrifuge tubes (~4 mL each) and absolute ethanol (16 mL) was added to each tube, shaken well and immersed in powdered dry ice for 1h. The tubes were centrifuged (20 °C, 3200 rpm, 20 min), and the clear solution decanted to afford crude product as white solid. The crude product was dissolved in distilled water (3.0 mL) and purified using Source-15Q ion exchange HPLC (0.5 mL injection, 0→5 min, 100% H_2O; 5→40 min, 100% H_2O to 100% 1M TEAB buffer, linear gradient @flow rate 6 mL/min). The compound eluting at 33 min (or 0.8M TEAB buffer) was collected and lyophilized to afford triethylammonium salt of triphosphate **4.65** as white solid (45 mg, 48%). ^{1}H NMR (300 MHz, D_2O) δ ppm 1.21 (t, J = 7.32 Hz, 36H, $HN(CH_2C\mathit{H}_3)_3$), 2.46 (dt, J = 14.42, 7.29 Hz, 1H, 2′-H), 2.66 (ddd, J = 14.13, 8.13, 3.22 Hz, 1H, 2′-H), 3.10 (q, J = 7.32 Hz, 25H, 3′-H &

$HN(CH_2CH_3)_3$), 3.95 (dd, *J* = 8.93, 6.30 Hz, 1H, 4′-H), 3.99 - 4.12 (m, 2H, 5′-H), 4.27 (dd, *J* = 8.79, 7.62 Hz, 1H, 4′-H), 6.37 (dd, *J* = 7.03, 3.22 Hz, 1 H), 8.16 (s, 1H, 2-H), 8.28 (s, 1H, 8-H). ^{13}C NMR (75 MHz, D_2O) δ ppm 8.44 ($HN(CH_2CH_3)_3$), 33.86 (2′-C), 38.35 (d, J_{p-c} = 8.29 Hz, 3′-C), 46.69 ($HN(CH_2CH_3)_3$), 66.86 (d, J_{p-c} = 6.08 Hz, 5′-C), 71.38 (4′-C), 85.31 (1′-C), 119.09 (5-C), 140.22 (8-C) 148.61 (4-C), 152.73 (2-C), 155.69 (6-C). ^{31}P NMR (121 MHz, D_2O) δ ppm -22.64 (dd, *J* = 21.11, 19.63 Hz, βP), -11.04 (d, *J* = 19.63 Hz, αP), -6.34 (d, *J* = 21.11 Hz, γP). ESI-HRMS $(M-H)^-$ calcd: 473.9981; found: 473.9982.

1′-(Thymin-1-yl)-5′-*O*-benzyl-β-D-apio-D-furanose (4.66): Using Vorbrüggen coupling condition-A and then following procedure described for **4.47**, compound **4.35** (500 mg, 1.36 mmol) rendered **4.66** (460 mg, 97%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.89 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 3.49 (d, *J* = 0.88 Hz, 1H, 3′-OH), 3.52 (s, 2H, 5′-H), 4.07 (d, *J* = 9.67 Hz, 1H, 4′-H_b), 4.24 (dd, *J* = 9.96, 0.88 Hz, 1H, 4′-H_a), 4.27 (dd, *J* = 5.57, 3.81 Hz, 1H, 2′-H), 4.38 (d, *J* = 4.10 Hz, 1H, 2′-OH), 4.56 (s, 2H, PhCH_2), 5.71 (d, *J* = 5.86 Hz, 1H, 1′-H), 7.22 (d, *J* = 1.17 Hz, 1H, 6-H), 7.27 - 7.40 (m, 5H, *Ph*CH_2), 9.18 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.51 (5-CH_3), 70.96 (5′-C), 73.69 (PhCH_2), 75.67 (4′-C), 76.91 (2′-C), 78.06 (3′-C), 92.38 (1′-C), 111.02 (5-C), 127.77, 128.04, 128.55, 137.41 (*Ph*CH_2), 135.54 (6-C), 151.47 (2-C), 163.74 (4-C). ESI-HRMS $[M+H]^+$ calcd, 349.1400; found, 349.1414.

1′-(Adenin-9-yl)-5′-(*O*-benzyl)-β-D-apio-D-furanose (4.67): Using Vorbrüggen coupling condition-B and then following procedure described for **4.48**, compound **4.35** (2.7 g, 7.37 mmol) rendered **4.67** (1.2 g, 46%) as white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.53 (q, *J* = 9.96 Hz, 2H, 5′-H), 3.83 (d, *J* = 9.08 Hz, 1H, 4′-H), 4.36 (d, *J* = 9.96 Hz, 1H, 4′-H), 4.58 (s, 2H, PhCH_2), 4.89 (t, *J* = 7.18 Hz, 1H, 2′-H), 5.08 (s, 1H, 3′-OH), 5.53 (d, *J* = 6.74 Hz, 1H, 2′-OH), 5.88 (d, *J* = 7.62 Hz, 1H, 1′-H), 7.19 - 7.29 (br.s, 2H, NH_2), 7.29 - 7.45 (m, 5H, *Ph*CH_2), 8.14 (s, 1H, 2-H), 8.34 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 71.28 (5′-C), 72.57 (PhCH_2), 73.78 (2′-C), 74.83 (4′-C), 77.45 (3′-C), 87.78 (1′-C), 119.40 (5-C), 127.29, 127.38, 128.24, 138.40 (*Ph*CH_2), 140.25 (8-C), 149.64 (4-C), 152.56 (2-C), 156.06 (6-C). ESI-HRMS $[M+H]^+$ calcd, 358.1515; found, 358.1516.

1′-(Thymin-1-yl)-2′,3′-(*O*-thiocarbonyl)-5′-(*O*-benzyl)-β-D-apio-D-furanose (4.68): To a solution of **4.66** (200 mg, 0.57 mmol) in DMF (4 mL) was added thiocarbonyldiimidazole (112 mg, 0.63 mmol) and the mixture heated to 80 °C for 90 minutes. The volatiles were removed under reduced pressure and the residue was purified by silica-gel column chromatography (2% MeOH in CH_2Cl_2) to afford the title thiacarbonate **4.68** (200 mg, 89%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.94 (d, *J* = 1.17 Hz, 3H, 5-C*H*$_3$), 3.89 (d, *J* = 11.13 Hz, 1H, 5′-H), 4.17 (d, *J* = 10.84 Hz, 1H, 5′-H), 4.30 - 4.42 (m, 2H, 4′-H), 4.57 - 4.71 (m, 2H, PhC*H*$_2$), 5.47 (d, *J* = 0.88 Hz, 1H, 1′-H), 5.82 (d, *J* = 1.17 Hz, 1H, 2′-H), 7.03 (d, *J* = 1.17 Hz, 1H, 6-H), 7.27 - 7.38 (m, 5H, *Ph*CH$_2$), 9.35 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.29 (5-*C*H$_3$), 67.73 (5′-C), 73.74 (Ph*C*H$_2$), 77.44 (4′-C), 88.71 (2′-C), 97.49 (1′-C), 100.15 (3′-C), 112.22 (5-C), 127.63, 127.99, 128.52, 137.05 (*Ph*CH$_2$), 139.36 (6-C), 151.18 (2-C), 163.57 (4-C), 189.42 (*C*S). ESI-HRMS $[M+H]^+$ calcd, 391.0964; found, 391.0544.

1′-(Adenin-9-yl)-2′,3′-(*O*-thiocarbonyl)-5′-(*O*-benzyl)-β-D-apio-D-furanose (4.69): Following the procedure described for the synthesis of **4.68**, compound **4.67** (300 mg, 0.84 mmol) rendered title compound **4.69** (260 mg, 78%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 4.03 (d, *J* = 10.84 Hz, 1H, 5′-H), 4.33 (d, *J* = 11.13 Hz, 1H, 4′-H), 4.42 (d, *J* = 11.13 Hz, 1H, 4′-H), 4.45 (d, *J* = 10.54 Hz, 1H, 5′-H), 4.61, 4.75 (d, *J* = 12.30 Hz, 2H, PhC*H*$_2$), 5.74 (br. s, 2H, NH_2), 6.14 (s, 1H, 2′-H), 6.20 (s, 1H, 1′-H), 7.29 - 7.40 (m, 5H, *Ph*CH$_2$), 7.87 (s, 1H, 8-H) 7.95 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 66.95 (5′-C), 73.86 (Ph*C*H$_2$), 75.00 (4′-C), 88.29 (2′-C), 90.33 (1′-C), 99.62 (3′-C), 119.90 (5-C), 127.98, 128.22, 128.63, 136.93 (*Ph*CH$_2$), 140.28 (8-C), 149.13 (4-C), 153.04 (2-C), 155.59 (6-C), 189.43 (*C*S). ESI-HRMS $[M+H]^+$ calcd, 400.1079; found, 400.1060.

1′-(Thymin-1-yl)-2′,3′-(dideoxydidehydro)-5′-(*O*-benzyl)-β-D-apio-D-furanose (4.70): A solution of compound **4.67** (180 mg, 0.46 mmol) in trimethyphosphite ($P(OCH_3)_3$, 8.0 mL) was heated to 120 °C for 6h. The volatiles materials were removed under reduced pressure and then co-evoporated 2-3 times with toluene. the residue was purified by silica-gel column chromatography (0-2% MeOH in CH_2Cl_2)

to afford **4.70** (130 mg, 90%) as a foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.91 (d, *J* = 1.17 Hz, 3H, 5-C*H*$_3$), 4.25 (s, 2H, 5′-H), 4.58 (s, 2H, PhC*H*$_2$), 4.63 - 4.74 (m, 1H, 4′-H), 4.77 - 4.90 (m, 1H, 4′-H), 5.67 - 5.76 (m, 1H, 2′-H) 6.91 (q, *J*=1.17 Hz, 1H, 6-H), 7.00 (m, 1H, 1′-H), 7.29 - 7.44 (m, 5H, *Ph*CH$_2$), 8.47 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.60 (5-*C*H$_3$), 65.08 (5′-*C*H$_2$), 73.18 (Ph*C*H$_2$), 75.57 (4′-C), 90.91 (1′-C), 111.26 (5-C), 119.90 (2′-C), 127.77, 128.11, 128.61, 137.28 (*Ph*CH$_2$), 135.33 (6-C), 145.44 (3′-C), 150.47 (2-C), 163.63 (4-C). ESI-HRMS $[M+Na]^+$ calcd, 337.1159; found, 337.1168.

1′-(Thymin-1-yl)-2′,3′-(dideoxy)-β/α-D-apio-D/L-furanose (4.1a + 4.61a): Following the procedure described for the synthesis of **4.52a**, compound **4.70** (120 mg, 0.38 mmol) rendered **4.1a** and **4.61a** as inseparable mixtures in 4: 1 ratio respectively (77 mg, 89%) as a white solid.

1′-(Adenin-9-yl)-β-D-apio-D-furanose (4.3b): Following the procedure described for the synthesis of **4.53b**, compound **4.67** (1.2g, 3.37 mmol) rendered title compound **4.3b** (800 mg, 89%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.46 (q, *J* = 11.13 Hz, 1H, 5′-H), 3.76 (d, *J* = 9.08 Hz, 1H, 4′-H), 4.31 (d, *J* = 9.37 Hz, 1H, 4′-H), 4.80 (t, *J* = 6.44 Hz, 1H, 2′-H), 4.85 (s, 1H, 3′-OH), 4.91 (br. s, 1H, 5′-OH), 5.42 (d, *J* = 6.44 Hz, 1H, 2′-OH), 5.88 (d, *J* = 7.62 Hz, 1H, 1′-H), 7.26 (s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.33 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 62.42 (5′-C), 73.37 (2′-C), 74.53 (4′-C), 78.23 (3′-C), 87.65 (1′-C), 119.27 (5-C), 139.93 (8-C), 149.72 (4-C), 152.62 (2-C), 156.04 (6-C). ESI-HRMS for $[M+H]^+$ calcd, 268.1046; found, 268.1107.

1,2-*O*-Isopropylidene-3-deoxy-β-D-apio-L-furanose (4.30): Following the procedure described for the synthesis of **4.52a**, compound **4.31** (3.7 g, 14 mmol) rendered **4.30** (2.2 g, 90%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.34 (d, *J* = 0.59 Hz, 3H, C(C*H*$_3$)$_2$), 1.53 (s, 3H, C(C*H*$_3$)$_2$), 2.21 (br. s, 1H, 5-OH), 2.34 (ddtd, *J* = 11.44, 6.94, 5.97, 5.97, 4.83 Hz, 1H, 3-H), 3.82 - 3.91 (m, 3H, 4-H & 5-H's), 3.97 (dd, *J* = 8.49, 7.32 Hz, 1H, 4-H), 4.73 (t, d, *J* = 4.39 Hz,1H, 2-H), 5.86 (d, *J* = 3.81 Hz, 1H, 1-H).

1,2-*O*-Isopropylidene-3-deoxy-α-D-apio-D-furanose (4.71): To a solution of compound **4.30** (2.2 g, 12.63 mmol) in 400 mL of acetone was added concentrated sulfuric acid (2.2 mL) and the mixture was stirred at room temperature for 1.5h. Then sodium carbonate (14 g) was added and stirred at room temperature for 45 minutes. Inorganic salts were removed by filtration and the filtrate concentrated under reduced pressure to afford oil. TLC indicated the conversion in favour of required isomer (roughly 2:1). The title compound is slightly more polar than the starting material (R_f after two runs: 0.35 for **4.71** and 0.4 for **4.30**; eluent, 2.5% MeOH in CH_2Cl_2). Silica-gel flash column chromatography (0.5-1.5% MeOH in CH_2Cl_2) afforded title compound and starting material. After three cycles 1.6 g (73%) of **4.71** was procured as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.31 (d, *J* = 0.59 Hz, 3H, C(C*H*$_3$)b), 1.50 (s, 3H, C(C*H*$_3$)a), 1.89 (br. s, 1H, 5-OH), 2.36 - 2.46 (m, 1H, 3-H), 3.58 (dd, *J* = 6.59, 3.37 Hz, 2H, 5-C*H*$_2$), 3.83 (d, *J* = 9.08 Hz, 1H, 4-Hb), 4.10 (dd, *J* = 8.93, 5.13 Hz, 1H, 4-Ha), 4.60 (d, *J* = 3.51 Hz, 1H, 2-H), 5.81 (d, *J* = 3.81 Hz, 1H, 1-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 26.20 (C(*C*H$_3$)b), 26.81 (C(*C*H$_3$)a), 48.07 (3-C), 62.00 (5-C), 68.74 (4-C), 82.28 (2-C), 105.61 (1-C), 111.25 (*C*(CH$_3$)$_2$).

1,2-*O*-Isopropylidene-3-deoxy-5-(*O*-benzyl)-α-D-apio-D-furanose (4.32): To an ice cold solution of compound **4.71** (1.6 g, 9.2 mmol) in DMF (30 mL) was added NaH (60% in mineral oil, 0.55g, 13.8 mmol) and then benzyl bromide (1.64 mL, 13.8 mmol) drop wise. The reaction mixture was stirred at room temperature overnight. Methanol (5 mL) was added and stirred further for 30 minutes. The volatile materials were removed under vacuo and the residue was partitioned between ethylacetate and water. The organic layer was separated, dried, evaporated and the residue purified by column chromatography (5-15% EtOAc in hexanes) to afford **4.32** (2.3 g, 95%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.31 (d, *J* = 0.59 Hz, 3H, C(C*H*$_3$)b), 1.51 (s, 3H, C(C*H*$_3$)a), 2.56 (td, *J* = 7.54, 5.13 Hz, 1H, 3-H), 3.37 (d, *J* = 7.62 Hz, 2H, 5-C*H*$_2$), 3.83 (d, *J* = 8.79 Hz, 1H, 4-Hb) 4.09 (dd, *J* = 8.93, 5.13 Hz, 1H, 4-Ha) 4.51 (d, *J* = 3.22 Hz, 2H, PhC*H*$_2$), 4.56 (d, *J* = 3.51 Hz, 1H, 2-H), 5.79 (d, *J* = 3.51 Hz, 1H, 1-H), 7.27 - 7.41 (m, 5H, *Ph*CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 26.30 (C(*C*H$_3$)b), 26.90 (C(*C*H$_3$)a), 46.07 (3-C), 68.78 (4&5-C), 73.34 (Ph*C*H$_2$), 82.39 (2-C), 105.55 (1-

C), 111.24 ($C(CH_3)_2$) 127.77, 127.90, 128.59, 138.06 ($Ph$$CH_2$). ESI-HRMS for $[M+K]^+$ calcd, 303.0999; found, 303.1078.

1,2-Di-*O*-acetyl-3-deoxy-5-(*O*-benzyl)-α/β-D-apio-D-furanose (4.72): Following the procedure described for the synthesis of **4.36**, compound **4.32** (1.3 g, 4.92 mmol) rendered **4.72** (1.2 g, 79%) as colorless oil. Mixture of α+β (3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.00 (s, major, $C(CH_3)_2$) 2.04 (s, minor, $C(CH_3)_2$) 2.07 (s, minor, $C(CH_3)_2$) 2.08 (s, major, $C(CH_3)_2$), 2.56 - 2.69 (m, major, 3-H) 2.69 - 2.83 (m, minor, 3-H) 3.46 - 3.74 (m, major & minor, 5-H) 3.80 - 3.94 (m, major & minor, 4-H) 4.20 – 4.34 (m, major & minor, 4-H) 4.51 (s, minor, $PhCH_2$), 4.54 (s, major, $PhCH_2$), 5.05 (t, J = 4.10 Hz, minor, 2-H), 5.08 (d, J = 2.64 Hz, major, 2-H), 6.13 (s, major, 1-H), 6.33 (d, J = 4.39 Hz, minor, 1-H), 7.27 - 7.40 (m, major & minor, $Ph$$CH_2$). ESI-HRMS for $[M+K]^+$ calcd, 347.0897; found, 347.0898.

1′-(Thymin-1-yl)-3′-deoxy-5′-*O*-benzyl-β-D-apio-D-furanose (4.73): Using Vorbrüggen coupling condition-A and then following procedure described for **4.47**, compound **4.72** (550 mg, 1.78 mmol) gave **4.73** (360 mg, 60%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.84 (d, J = 0.7 Hz, 3H, 5-CH_3), 2.68 (ddt, J = 12.72, 7.65, 6.41, 6.41 Hz, 1H, 3′-H), 3.51 (dd, J = 9.51, 6.64 Hz, 1H, 5′-H), 3.59 (dd, J = 9.51, 5.02 Hz, 1H, 5′-H), 4.01 (dd, J = 8.79, 7.89 Hz, 1H, 4′-H), 4.22 (dd, J = 6.19, 3.86 Hz, 1H, 2′-H), 4.32 (dd, J = 8.79, 7.89 Hz, 1H, 4′-H), 4.50 (s, 2H, $PhCH_2$), 5.60 (d, J = 3.77 Hz, 1H, 1′-H), 7.24 (d, J = 1.26 Hz, 1H, 6-H), 7.26 - 7.38 (m, 5H, $Ph$$CH_2$), 9.77 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.53 (5-CH_3), 46.38 (3′-C), 68.58 (5′-C), 71.44 (4′-C), 73.31 ($PhCH_2$), 79.23 (2′-C), 94.34 (1′-C), 110.43 (5-C), 127.66, 127.88, 128.48 ($Ph$$CH_2$) 134.98 (6-C) 137.78 ($Ph$$CH_2$), 151.58 (2-C), 164.11 (4-C). ESI-HRMS for $[M+H]^+$ calcd, 333.1445; found, 333.1452.

1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-benzyl-β-D-apio-D-furanose (4.74): Using Vorbrüggen coupling condition-B and then following procedure described for **4.48**, compound **4.72** (1.55 g, 5 mmol) gave **4.74** (480 mg, 28%) and its α-anomer (200 mg, 11%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.60 (quind, J = 8.13, 4.98 Hz, 1H, 3′-H), 3.61 (t, J = 8.49 Hz, 1H, 5′-H), 3.70 (dd, J = 9.67, 4.98 Hz, 1H, 5′-

H), 4.05 (t, *J* = 8.79 Hz, 1H, 4′-Hb), 4.17 (t, *J* = 8.20 Hz, 1H, 4′-Ha), 4.51 (s, 2H, PhC*H*$_2$), 4.70 (dt, *J* = 7.62, 5.71 Hz, 1H, 2′-H), 5.69 (d, *J* = 5.86 Hz, 1H, 2′-OH), 5.79 (d, *J* = 5.56 Hz, 1H, 1′-H), 7.26 (s, 2H, NH$_2$), 7.29 - 7.40 (m, 5H, *Ph*CH$_2$), 8.13 (s, 1H, 2-H), 8.31 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 46.65 (3′-C), 69.19 (5′-C), 70.45 (4′-C), 72.16 (Ph*C*H$_2$), 75.12 (2′-C), 89.96 (1′-C), 119.23 (5-C), 127.44, 127.46, 128.27, 138.34 (*Ph*CH$_2$), 139.79 (8-C), 149.42 (4-C), 152.57 (2-C), 156.05 (6-C). ESI-HRMS for [M+H]$^+$ calcd, 342.1566; found, 342.1553. Spectral data for **1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-benzyl-α-D-apio-D-furanose.** ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.69 - 2.83 (m, 1H, 3′-H), 3.55 (dd, *J* = 9.52, 7.18 Hz, 1H, 5′-H), 3.66 (dd, *J* = 9.52, 5.13 Hz, 1H, 5′-H), 3.73 (dd, *J* = 8.49, 7.03 Hz, 1H, 4′-Hb), 4.29 (q, *J* = 5.56 Hz, 1H, 2′-H), 4.36 (t, *J* = 8.20 Hz, 1H, 4′-Ha), 4.54 (s, 2H, PhC*H*$_2$), 5.53 (d, *J* = 5.27 Hz, 1H, 2′-OH), 6.19 (d, *J* = 5.27 Hz, 1H, 1′-H), 7.22 (s, 2H, NH$_2$), 7.26 - 7.43 (m, 5H, *Ph*CH$_2$), 8.14 (s, 1H, 2-H), 8.16 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 45.32 (3′-C), 69.17 (4′-C), 69.42 (5′-C), 71.98 (2′-C) 72.22 (Ph*C*H$_2$), 84.36 (1′-C), 118.23 (5-C), 127.48, 127.54, 128.30, 138.33 (*Ph*CH$_2$), 140.21 (8-C), 149.55 (4-C), 152.35 (2-C), 155.84 (6-C).

1′-(Thymin-1-yl)-3′-deoxy-β-D-apio-D-furanose (4.2a): Following the procedure described for the synthesis of **4.52a**, compound **4.73** (350 mg, 1.05 mmol) gave **4.2a** (220 mg, 86%) as white solid. ^{1}H NMR (300 MHz, CD$_3$OD) δ ppm 1.89 (d, *J* = 1.17 Hz, 3H, 5-CH$_3$) 2.39 - 2.55 (m, 1H, 3′-H), 3.65 (dd, *J* = 10.84, 6.74 Hz, 1H, 5′-H), 3.73 (dd, *J* = 10.98, 4.83 Hz, 1H, 5′-H), 4.02 - 4.10 (t, *J* = 8.20 Hz, 1H, 4′-H), 4.17 - 4.26 (m, 2H, 2′ & 4′-H's), 5.72 (d, *J* = 5.56 Hz, 1H, 1′-H), 7.46 (d, *J* = 1.17 Hz, 1H, 6-H). ^{13}C NMR (75 MHz, CD$_3$OD) δ ppm 11.16 (5-*C*H$_3$), 48.29 (3′-C), 60.46 (5′-C), 70.25 (4′-C), 75.70 (2′-C), 92.29 (1′-C), 110.42 (5-C), 137.14 (6-C), 151.56 (2-C), 165.23 (4-C). ESI-HRMS for [M+H]$^+$ calcd, 243.0981; found, 243.0975.

1′-(Adenin-9-yl)-3′-deoxy-β-D-apio-D-furanose (4.2b): Following the procedure described for the synthesis of **4.52b**, compound **4.74** (600 mg, 1.76 mmol) gave **4.2b** (390 mg, 88%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.34 - 2.48 (m, 1H, 3′-H), 3.56 (dd, *J* = 10.69, 7.76 Hz, 1H, 5′-H), 3.68 (dd, *J* = 10.69, 4.54 Hz, 1H, 5′-H), 4.04 (t, *J* = 8.79 Hz, 1H, 4′-H), 4.13 (t, *J* = 8.20 Hz, 1H, 4′-H), 4.62 (t, *J* = 6.44

Hz, 1H, 2′-H), 4.79 (br.s, 1H, 5′-OH), 5.61 (br.s, 1H, 2′-OH), 5.79 (d, *J* = 5.56 Hz, 1H, 1′-H), 7.26 (s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.31 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 48.98 (3′-C), 60.18 (5′-C), 70.29 (4′-C), 75.12 (2′-C), 89.98 (1′-C), 119.15 (5-C), 139.61 (8-C), 149.46 (4-C), 152.59 (2-C), 156.04 (6-C). ESI-HRMS for $[M+H]^+$ calcd, 252.1097; found, 252.1081.

1′-(Thymin-1-yl)-3′-deoxy-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (4.75): Following a similar procedure described for compound **4.54**, compound **4.2a** (200 mg, 0.83 mmol) afforded compound **4.75** (260 mg, 88%) as a foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.05 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 1.92 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.51 - 2.66 (m, 1H, 3′-H), 3.69 (dd, *J* = 10.54, 6.15 Hz, 1H, 5′-H), 3.75 (dd, *J* = 10.25, 4.69 Hz, 1H, 5′-H), 3.94 - 4.07 (m, 2H, 4′-H & 2′-OH), 4.18 (ddd, *J* = 6.96, 4.03, 2.78 Hz, 1H, 2′-H), 4.28 (t, *J* = 8.35 Hz, 1H, 4′-H), 5.61 (d, *J* = 4.10 Hz, 1H, 1′-H), 7.27 (d, *J* = 1.17 Hz, 1H, 6-H), 9.42 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.40, -5.35 ($SiCH_3$), 12.71 (5-CH_3), 18.34 ($C(CH_3)_3$), 25.92 ($C(CH_3)_3$), 48.36 (3′-C), 60.80 (5′-C), 70.95 (4′-C), 78.74 (2′-C), 94.35 (1′-C), 110.69 (5-C), 134.88 (6-C), 151.72 (2-C), 164.07 (4-C). ESI-HRMS for $[M+H]^+$ calcd, 357.1846; found, 357.1855.

1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (4.76): Following a similar procedure described for compound **4.54**, compound **4.2b** (350 mg, 1.39 mmol) afforded compound **4.76** (415 mg, 82%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.03, 0.04 (s's, 2 x 3H, $Si(CH_3)_3$), 0.85 (s, 9H, $C(CH_3)_3$), 2.64 - 2.79 (m, 1H, 3′-H), 3.76 (dd, *J* = 10.40, 6.30 Hz, 1H, 5′-H), 3.85 (dd, *J* = 10.40, 4.54 Hz, 1H, 5′-H), 4.15 (t, *J* = 9.08 Hz, 1H, 4′-H), 4.30 - 4.40 (t, *J* = 8.49 Hz, 1H, 4′-H), 4.52 (dd, *J* = 8.64, 5.71 Hz, 1H, 2′-H), 5.69 (br.s, 1H, 2′-OH), 5.79 (d, *J* = 5.86 Hz, 1H, 1′-H), 5.95 (s, 2H, NH_2), 7.97 (s, 1H, 8-H), 8.27 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.53, -5.49 ($SiCH_3$), 18.24 ($C(CH_3)_3$), 25.80 ($C(CH_3)_3$), 47.70 (3′-C), 61.06 (5′-C), 71.08 (4′-C), 77.36 (2′-C), 92.83 (1′-C), 120.08 (5-C), 138.38 (8-C), 149.18 (4-C), 152.51 (2-C), 155.53 (6-C). ESI-HRMS for $[M+H]^+$ calcd, 366.1961; found, 366.1962.

1′-(Thymin-1-yl)-2′,3′-dideoxy-5′-(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (4.77): Following a similar procedure described for compound **4.56**, compound **4.75** (250 mg, 0.70 mmol) gave compound **4.77** (215 mg, 90 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.06 (s, 6H, SiCH_3), 0.89 (s, 9H, C(CH_3)$_3$), 1.77 (ddd, *J* = 13.25, 8.86, 7.18 Hz, 1H, 2′-H), 1.94 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.43 - 2.55 (m, 1H, 2′-H), 2.55 - 2.72 (m, 1H, 3′-H), 3.60 (dd, *J* = 10.25, 5.86 Hz, 1H, 5′-H), 3.67 (dd, *J* = 10.25, 4.98 Hz, 1H, 5′-H), 3.94 (t, *J* = 7.80 Hz, 1H, 4′-H), 4.07 (t, *J* = 8.05 Hz, 1H, 4′-H), 6.06 (dd, *J* = 7.03, 6.44 Hz, 1H, 1′-H), 7.21 (q, *J* = 1.18 Hz,1H, 6-H), 8.31 (br.s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.47, -5.44 (SiCH_3), 12.62 (5-CH_3), 18.25 (C(CH$_3$)$_3$), 25.82 (C(CH$_3$)$_3$), 34.57 (2′-C), 40.88 (3′-C), 62.64 (5′-C), 71.02 (4′-C), 86.63 (1′-C), 110.87 (5-C), 134.93 (6-C), 150.34 (2-C), 163.79 (4-C). ESI-HRMS for $[M+H]^+$ calcd, 341.1897; found, 341.1891.

1′-(Adenin-9-yl)-2′,3′-dideoxy-5′-(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (4.78): Following a similar procedure described for compound **4.56**, compound **4.76** (400 mg, 1.10 mmol) gave compound **4.78** (310 mg, 81 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.05 (s, 6H, $SiCH_3$), 0.88 (s, 9H, C(CH$_3$)$_3$), 2.33 - 2.50 (m, 1H, 2′-H), 2.57 - 2.81 (m, 2H, 2′ & 3′-H's), 3.71 (d, *J* = 5.27 Hz, 2H, 5′-H), 4.04 (t, *J* = 8.20 Hz,1H, 4′-H), 4.14 (t, *J* = 7.59 Hz, 1H, 4′-H), 5.82 (br.s, 2H, NH_2), 6.29 (t, *J* = 5.86 Hz,1H, 1′-H), 8.05 (s, 1H, 8-H), 8.36 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.44 ($SiCH_3$), 18.29 (C(CH$_3$)$_3$), 25.85 (C(CH$_3$)$_3$)' 34.56 (2′-C), 41.59 (3′-C), 63.00 (5′-C), 71.09 (4′-C), 85.50 (1′-C), 120.22 (5-C), 138.43 (8-C), 149.71 (4-C), 153.00 (2-C), 155.46 (6-C). ESI-HRMS for $[M+H]^+$ calcd, 350.2012; found, 350.2009.

1′-(Thymin-1-yl)-2′,3′-dideoxy-β-D-apio-D-furanose (4.1a): Following a similar procedure described for the synthesis of compound **4.61b**, compound **4.77** (200 mg, 0.59 mmol) gave compound **4.1a** (115 mg, 86 %) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.71 (t, *J* = 4.69 Hz, 1H, 5′-OH), 1.74 - 1.86 (m, 1H, 2′-H), 1.94 (d, *J* = 1.46 Hz, 3H, 5-CH_3), 2.51 - 2.75 (m, 2H, 2′ & 3′-H's), 3.64 - 3.81 (m, 2H, 5′-H), 3.98 (dd, *J* = 8.79, 7.03 Hz, 1H, 4′-H), 4.07 - 4.16 (m, 1H, 4′-H), 6.02 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.27 - 7.30 (q, *J* = 1.44 Hz, 1H, 6-H), 8.43 (br.s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 12.64 (5-CH_3), 34.70 (2′-C), 40.65 (3′-C), 63.23 (5′-C), 71.16 (4′-C),

86.92 (1′-C), 110.78 (5-C), 135.28 (6-C), 150.26 (2-C), 163.63 (4-C). ESI-HRMS for $[M-H]^-$ calcd, 225.0881; found, 225.0875.

1′-(Adenin-9-yl)-2′,3′-dideoxy-α-D-apio-L-furanose (4.1b): Following a similar procedure described for the synthesis of compound **4.61b**, compound **4.78** (300 mg, 0.86 mmol) gave compound **4.1b** (190 mg, 94 %) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.25 - 2.39 (m, 1H, 2′-H), 2.52 - 2.67 (m, 2H, 2′ & 3′-H's), 3.48 - 3.65 (m, 2H, 5′-H), 3.89 (t, J = 8.20 Hz, 1H, 4′-H), 4.00 (t, J = 7.91 Hz, 1H, 4′-H), 4.82 (t, J = 5.13 Hz, 1H, 5′-OH), 6.23 (t, J = 6.74 Hz, 1H, 1′-H), 7.26 (s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.32 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 33.73 (2′-C), 41.70 (3′-C), 61.67 (5′-C), 70.77 (4'-C), 84.28 (1′-C), 119.17 (5-C), 139.05 (8-C), 149.17 (4-C), 152.52 (2-C). 156.02 (6-C). ESI-HRMS for $[M+H]^+$ calcd, 236.1147; found, 236.1137.

1′-(Adenin-9-yl)-2′,3′-dideoxy-β-D-apio-D-furanose triphosphate (4.79): following the synthetic protocol described for **4.65**, compound **4.1b** (25 mg, 0.106 mmol) gave triphosphate derivative **4.79** (17 mg, 21%) as highly hygroscopic colorless solid. ^{1}H NMR (300 MHz, D_2O) δ ppm 1.27 (t, J = 7.32 Hz, 24H, NCH_2CH_3), 1.33 (t, J = 7.32 Hz, 3H, NCH_2CH_3), 2.42 (ddd, J = 13.62, 8.64, 6.74 Hz, 1H, 2′-H), 2.74 - 2.89 (m, 1H, 2′-H), 2.89 - 3.12 (m, 3H, 3′-H & NCH_2CH_3), 3.19 (q, J = 7.32 Hz, 14H, NCH_2CH_3), 3.54 (q, J = 7.13 Hz, 2H, NCH_2CH_3), 4.05 (t, J = 8.64 Hz, 1H, 4′-H), 4.14 (app-t, J = 6.15 Hz, 2H, 5′-H), 4.28 (t, J = 8.49 Hz, 1H, 4′-H), 6.35 (t, J = 6.74 Hz, 1H, 1′-H), 8.26 (s, 1H), 8.47 (s, 1H). ^{13}C NMR (75 MHz, D_2O) δ ppm 7.34, 8.38, 10.68 (NCH_2CH_3), 33.59 (2′-C), 39.30 (d, J_{p-c} = 8.10 Hz, 3′-C), 42.36, 46.79 (NCH_2CH_3), 66.32 (d, J_{p-c} = 5.92 Hz, 5′-C), 70.79 (4′-C), 85.05 (1′-C), 150.79, 154.26. ^{31}P NMR (121 MHz, D_2O) δ ppm -23.28 (br. s, 1P, β-P) -11.20, -11.04 (br. d, 2P, α & γ-P). ESI-HRMS for $[M-H]^-$ calcd, 473.9981; found, 473.9987.

Phenyl(isopropoxy-L-alaninyl)phosphorochloridate (4.60): To a stirred solution of phenyldichlorophosphate (0.30 mL, 2.00 mmol), L-alanine isopropyl ester hydrochloride (0.34 g, 2.00 mmol) in anhydrous CH_2Cl_2 (15 mL), anhydrous TEA (0.56 mL, 4.00 mmol) was added dropwise under an argon atmosphere at -78 ^{o}C.

Following the addition the reaction mixture was stirred at -78 ^{o}C for 30 min, then at room temperature for 2h. Formation of the desired compound was monitored by ^{31}P NMR. After this period the solvent was removed under reduced pressure and the residue triturated with anhydrous diethyl ether. The precipitate was filtered under nitrogen and the solution was concentrated to give **4.60** (0.58 g, 96%) as a yellow oil. ^{31}P NMR ($CDCl_3$, 202 MHz): δ 8.13, 7.75. ^{1}H NMR ($CDCl_3$, 500 MHz): δppm 7.47-7.16 (m, 5H, PhO), 5.18-4.98 (m, 1H, COOCH), 4.41, 4.33 (2bs, 1H, N*H*CH), 4.21-4.09 (m, 1H, NHC*H*), 1.53, 1.51 (2d, 3H, *J* = 2.30, CHCH_3), 1.35-1.27 (m, 6H, COOCH(CH_3)$_2$).

1′-(Thymin-1-yl)-2′,3′-dideoxy-α-D-apio-D-furanose [phenyl-(isopropoxy-L-alaninyl)] phosphate (4.80a): To a solution of **4.1a** (0.050 g, 0.22 mmol) in anhydrous THF (4 mL) was added a solution of phosphorochloridate **4.60** (0.203g, 0.66 mmol) in anhydrous THF (2 mL), followed by drop wise addition, under an argon atmosphere, of anhydrous N-methylimidazole (0.88 mL, 1.11 mmol) and the reaction mixture was stirred at room temperature for 48h. After this period, the solvent was removed and the residue taken up in dichloromethane and washed with 0.5 M HCl (2 x 15 mL). The combined organics were dried over $MgSO_4$ filtered and evaporated. The residue was purified by preparative thin layer chromatography (2000 micron, Aldrich) using a mixture DCM/MeOH 95:5 v/v as eluent to give a **4.80a** (0.096 g, 88%) as a pale yellow foamy solid. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.58, 7.57 (2s, 2H, H-6), 7.45 (d, *J* = 8.0 Hz, 2H, Ph), 7.44 (d, *J* = 7.5 Hz, 2H, Ph), 7.33-7.28 (m, 6H, Ph), 6.10 (t, *J* = 7.0 Hz, 1H, H-1′), 6.08 (t, *J* = 7.0 Hz, 1H, H-1′), 5.10-5.03 (m, 2H, C*H*(CH_3)$_2$), 4.33-4.21 (m, 4H, CH_2OP), 4.16-4.10 (m, 2H, CH_2O), 4.01-3.96 (m, 2H,CH_2O), 3.94-3.89 (m, 2H, C*H*CH_3), 2.95-2.86 (m, 2H, H-3′), 2.64-2.56 (m, 2H, H-2′a), 1.97 (s, 6H, CH_3), 1.95-1.88 (m, 2H, H-2′b), 1.43 (d, *J* = 7.5 Hz, 3H, CHCH_3), 1.40 (d, *J* = 6.5 Hz, 3H, CHCH_3), 1.34-1.30 (m, 12H, CH(CH_3)$_2$). ^{13}C NMR (125 MHz, CD_3OD) δ ppm (d, J_{CP} = 5.4 Hz, CO_2*i*Pr), 174.54 (d, J_{CP} = 4.5 Hz, CO_2iPr), 166.46, 166.44 (CO), 152.30 (d, J_{CP} = 3.6 Hz, C_{ipso}OPh), 152.28 (CO), 152.25 (d, J_{CP} = 3.6 Hz, C_{ipso}OPh), 137.57 (C-6), 130.84, 130.81, 126.25, 126.23 (Ph), 121.54 (d, J_{CP} = 4.5 Hz, Ph), 121.47 (d, J_{CP} = 5.3 Hz, Ph), 111.69 (C-5), 88.15, 88.12, (C-1′), 71.73, 71.58 (CH_2O), 70.19, 70.16 (*C*H(CH_3)$_2$), 68.42 (d, J_{CP} = 5.4 Hz,

CH_2OP), 68.32 (d, J_{CP} = 5.4 Hz, CH_2OP), 51.89, 51.88 ($CHCH_3$), 40.55 (d, J_{CP} = 3.5 Hz, C-3′), 40.49 (d, J_{CP} = 3.6 Hz, C-3′), 35.27, 35.18 (CH_2), 22.05, 22.03, 21.98, ($CH(CH_3)_2$), 20.55 (d, J_{CP} = 7.2 Hz, $CHCH_3$), 20.43 (d, J_{CP} = 7.2 Hz, $CHCH_3$), 12.54, 12.52 (CH_3). ^{31}P NMR (202 MHz, CD_3OD) δ ppm 3.89, 3.49. ESI-MS; 518 $[M+Na]^+$. HPLC; ACN/H_2O 10:90 v/v to 100:0 in 30 min.; λ = 280 nm, flow 1 mL/min, t_R = 13.79, 13.81 min.

1′-(Adenin-9-yl)-2′,3′-dideoxy-α-D-apio-D-furanose [phenyl-(isopropoxy-L-alaninyl)] phosphate (4.80b): Following the reaction protocol mentioned for the synthesis of compound **4.80a**, **4.1b** (0.050 g, 0.21 mmol) was reacted with phosphorochloridate **4.60** (0.201g, 0.66 mmol) to give **4.80b** (0.054 g, 51%) as a white foamy solid. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.27, 8.25 (2s, 2H, H-8), 8.23, 8.22 (2s, 2H, H-2), 7.35 (d, *J* = 8.0 Hz, 2H, Ph), 7.34 (d, *J* = 7.8 Hz, 2H, Ph), 7.26-7.16 (m, 6H, Ph), 6.29 (t, *J* = 7.0 Hz, 1H, H-1′), 6.28 (t, *J* = 7.0 Hz, 1H, H-1′), 5.02-4.94 (m, 2H, $CH(CH_3)_2$), 4.33 (m, 4H, CH_2OP), 4.16-4.05 (m, 4H, CH_2O), 3.93-3.88 (m, 2H, $CHCH_3$), 2.96-2.89 (m, 2H, H-3′), 2.75- 2.66 (m, 2H, H2′a), 2.49-2.43 (m, 2H, H2′b), 1.35 (d, *J* = 7.0 Hz, 3H, CH_3), 1.33 (d, *J* = 7.0 Hz, 3H, CH_3), 1.23-1.21 (m, 12H, $CH(CH_3)_2$). ^{13}C NMR (125 MHz, CD_3OD) δ ppm (d, J_{CP} = 4.5 Hz, CO_2iPr), 174.51 (d, J_{CP} = 4.5 Hz, CO_2iPr), 157.35 (C-6), 153.89 (C-2), 152.27 (d, J_{CP} = 3.4 Hz, C_{ipso}OPh), 152.22 (d, J_{CP} = 2.6 Hz, C_{ipso}OPh), 150.38 (C-4), 140.82, 140.79 (C-8), 130.81, 126.19, 126.16 (Ph), 121.53 (d, J_{CP} = 5.5Hz, Ph), 121.45 (d, J_{CP} = 5.5Hz,CH Ph), 120.73, 120.70 (C-5), 87.04, 87.04, (C-1′), 71.89, 71.83 (CH_2O), 70.15 ($CH(CH_3)_3$), 68.32 (d, J_{CP} = 6.4 Hz, CH_2OP), 68.23 (d, J_{CP} = 6.4 Hz, CH_2OP), 51.88, 51.72 ($CHCH_3$), 41.19 (d, J_{CP} = 7.2 Hz, C-3′), 41.13 (d, J_{CP} = 7.2 Hz, C-3′), 35.24, 35.11 (CH_2), 22.00, 21.95, 21.94, ($CH(CH_3)_3$), 20.55 (d, J_{CP} = 6.4 Hz, $CHCH_3$), 20.43 (d, J_{CP} = 6.4 Hz, $CHCH_3$). ^{31}P NMR (202 MHz, CD_3OD) δ ppm 3.81, 3.46. ESI-MS; 505 $[M+H]^+$, 527 $[M+Na]^+$. HPLC; ACN/H_2O 10/90 v/v to 100/0 in 30 min, λ = 280 nm, flow 1 mL/min, t_R = 12.61 min.

1′-(Thymin-1-yl)-2′,3′-dideoxy-α-D-apio-D-furanose [phenyl-(benzoxy-L-alaninyl)] phosphate (4.6a): Following the reaction protocol mentioned for the synthesis of compound **4.80a**, **1a** (0.048 g, 0.21 mmol) was reacted with

phosphorochloridate **3.33** (0.22g, 0.64 mmol) to give **4.6a** (0.040 g, 35%) as a pale white foamy solid. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.47, 7.46 (d, *J* = 2.5Hz, 2H, H-6), 7.37-7.32 (m, 14H, Ph and CH_2*Ph*), 7.23-7.18 (m, 6H, Ph), 5.99 (t, *J* = 6.0 Hz, 1H, H-1′), 5.98 (t, *J* = 6.0 Hz, 1H, H-1′), 5.17-5.15 (m, 4H, C*H*$_2$Ph), 4.17-4.05 (m, 4H,CH_2OP), 4.04-4.01 (m, 2H, C*H*CH_3), 4.00-3.87 (m, 4H, CH_2O), 2.79-2.73 (m, 1H, H-3′), 2.72-2.66 (m, 1H, H-3′), 2.05-2.39 (m, 2H, H-2′a), 1.89, 1.88 (d, *J* = 1.5 Hz, 6H, CH_3), 1.81-1.72 (m, 2H, H-2′b), 1.38 (d, *J* = 7.5 Hz, 3H, CHC*H*$_3$), 1.35 (d, *J* = 7.5 Hz, 3H, CHC*H*$_3$). ^{13}C NMR (125 MHz, CD_3OD) δ ppm 174.93 (d, J_{CP} = 5.0 Hz, CO_2Bn), 174.74 (d, J_{CP} = 5.0 Hz, CO_2Bn), 166.44, 166.42 (CO), 152.30, 152.29 (CO), 152.21 (d, J_{CP} = 2.75 Hz, C_{ipso}OPh), 152.16 (d, J_{CP} = 2.75 Hz, C_{ipso}OPh), 137.54, 137.52 (C-6), 137.32, 137.31 (*C*$_{ipso}$OCH$_2$Ph), 130.82, 130.80 (Ph), 129.66, 129.64, 129.43, 129.40, 129.36, 129.31 (CH_2*Ph*), 126.25, 126.23 (Ph), 121.53 (d, J_{CP} = 4.6 Hz, Ph), 121.44 (d, J_{CP} = 5.3 Hz, Ph), 111.65 (C-5), 88.07, 88.03, (C-1′), 71.65, 71.46 (CH_2O), 68.37 (d, J_{CP} = 5.0 Hz, CH_2OP), 68.25 (d, J_{CP} = 5.0 Hz, CH_2OP), 67.98 (*C*H$_2$Ph), 51.83, 51.65 (*C*HCH$_3$), 40.45 (d, J_{CP} = 3.75 Hz, C-3′), 40.39 (d, J_{CP} = 3.75 Hz, C-3′), 35.20, 35.12 (CH_2), 20.35 (d, J_{CP} = 7.5 Hz, CH*C*H$_3$), 20.29 (d, J_{CP} = 7.5 Hz, CH*C*H$_3$), 12.51 (CH_3). ^{31}P NMR (202 MHz, CD_3OD) δ ppm 3.88, 3.33. MS (ESI); 566 $[M+Na]^+$. HPLC; ACN/H_2O 10/90 v/v to 100/0 in 30 min, λ = 280 nm, flow 1 mL/min, t_R = 15.42 min.

1′-(Adenin-9-yl)-2′,3′-dideoxy-α-D-apio-D-furanose [phenyl-(benzoxy-L-alaninyl)] phosphate (4.6b): Following the reaction protocol mentioned for the synthesis of compound **4.80a**, **4.1b** (0.050 g, 0.21 mmol) was reacted with phosphorochloridate **3.33** (0.23 g, 0.66 mmol) to give **4.6b** (0.030 g, 26%) as a white foamy solid. ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.24, 8.23 (2s, 1H, H-8), 8.22, 8.21 (2s, 1H, H-2), 7.40-7.26 (m, 16H, Ph), 7.22-7.15 (m, 4H, Ph), 6.27 (t, *J* = 7.0 Hz, 1H, H-1′), 6.24 (t, *J* = 6.5 Hz, 1H, H-1′), 5.14 (s, 4H, C*H*$_2$Ph), 4.26-4.19 (m, 4H CH_2OP), 4.10-3.96 (m, 6H, CH_2O and C*H*CH_3), 2.91-2.75 (m, 2H, H-3′), 2.69- 2.57 (m, 2H, H2′a), 2.41-2.34 (m, 2H, H2′b), 1.37 (d, *J* = 6.5 Hz, 3H, CH_3), 1.35 (d, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD) δppm 174.96 (d, J_{CP} = 4.2 Hz, CO_2Bn), 174.74 (d, J_{CP} = 4.6 Hz, CO_2Bn), 157.32 (C-6), 153.84, 153.83 (C-2), 152.22 (d, J_{CP} = 2.5 Hz, C_{ipso}OPh), 152.17 (d, J_{CP} = 2.5 Hz, C_{ipso}OPh), 150.36 (C-4), 140.78, 140.74 (C-8), 137.29, 137.28 (C_{ipso}OCH$_2$*Ph*), 130.80 (d, J_{CP} = 0.7 Hz, Ph), 130.78 (d, J_{CP} = 0.9 Hz, Ph), 129.60,

129.38, 129.36, 129.35, 129.30 (CH_2*Ph*), 126.20 (d, J_{CP} = 1.25 Hz, Ph), 126.17 (d, J_{CP} = 1.25 Hz, Ph), 121.52 (d, J_{CP} = 4.6 Hz, Ph), 121.42 (d, J_{CP} = 4.6 Hz, Ph), 120.70, 120.68 (C-5), 87.00, 86.98, (C-1′), 71.82, 71.70 (CH_2O), 68.22 (d, J_{CP} = 5.5 Hz, CH_2OP), 68.15 (d, J_{CP} = 5.5 Hz, CH_2OP), 67.99, 67.97 (*C*H_2Ph), 51.83 (d, J_{CP} = 1.4 Hz, *C*HCH_3), 51.65 (*C*HCH_3), 41.10 (d, J_{CP} = 7.8 Hz, C-3′), 41.05 (d, J_{CP} = 7.8 Hz, C-3′), 35.23, 35.10 (CH_2), 20.39 (d, J_{CP} = 7.0 Hz, CH*C*H_3), 20.33 (d, J_{CP} =7.0 Hz, CH*C*H_3). ^{31}P NMR (202 MHz, CD_3OD) δppm 3.80, 3.28. ESI-MS; 553 $[M+H]^+$, 575 $[M+Na]^+$. HPLC; ACN/H_2O 10/90 v/v to 100/0 in 30 min, λ = 280 nm, flow 1 mL/min, t_R = 14.36 min.

4.5.2. Pharmacological assay procedures

For other biological assays please refer to Chapter 3, Section 3.4.2

HIV-RT primer-template assay

Primer oligonucleotides 5′ CAGGAAACAGCTATGAC 3′ (Sigma Genosys) were labeled with 5′ [γ-^{33}P]-ATP (Perkin Elmer) using T4 polynucleotide kinase (New England Biolabs) according to the manufacturer's protocol. The labeled primers were further purified using illustra MicroSpin G-25 Column (GE Healthcare) and then annealed with template oligonucleotides 5′ TTTTTTTGTCATAGCTGTTTCCTG 3′ (Eurogentec) in a 1:2 molar ratio by heating the mixture at 75°C for 5 min, followed by slowly cooling to room temperature. The DNA polymerization mixtures containing 125 nM primer-template complex, reaction buffer (supplied with the HIV RT), 125, 500, or 1000 µM of modified triphosphate (**4.65**, **79**) and 0.03 U.µL^{-1} HIV RT (Ambion) were incubated at 37°C and aliquots were taken after 15, 30 and 60 min. In the control reaction, 50µM of natural dATP was used. All polymerase reactions were then stopped by adding a double volume of gel loading buffer (90% formamide, 50mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol). Samples were heated at 70°C for 5 min prior to separation on a 0.4mm 20% denaturing polyacrylamide gel. The bands were then visualized using phosphorimaging.

CHAPTER – 5

APIOADENOSINES AS A_3 ADENOSINE RECEPTOR MODULATORS

5.1. Objectives

Besides adenosine itself, which is used clinically for the treatment of supraventricular tachycardia and in myocardial perfusion imaging, only one adenosine receptor-specific agent, the A_{2A} AR agonist regedenoson, has so far been approved by the FDA. However, a relatively large group of AR ligands are currently under clinical evaluation (see Chapter 1, Section 1.4).

With the exception of compound **5.1**, which was reported in the mid 1980s as being inactive at A_1 and A_2ARs,[179] the recently reported carbocyclic analogue **5.2**,[180] a weak A_3AR agonist, 4′-hydroxymethyl transposed nucleosides have not been investigated as adenosine receptor ligands.

This led us to employ a new and convenient method for the synthesis of apioadenosines from 1,2-*O*-isopropylidene-α-L-threose (see Chapter 4) for the construction of suitably modified 9-(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenines as potential A_3AR modulators (Figure 1).

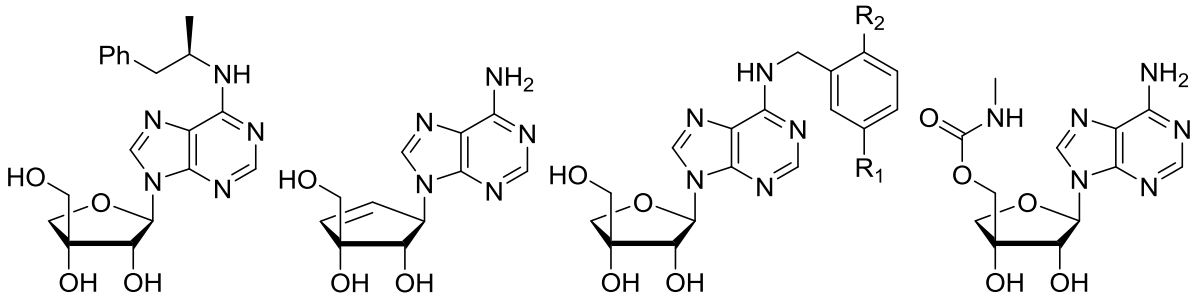


Figure 5.1. Known A_3AR modulators and target analogues in the present study.

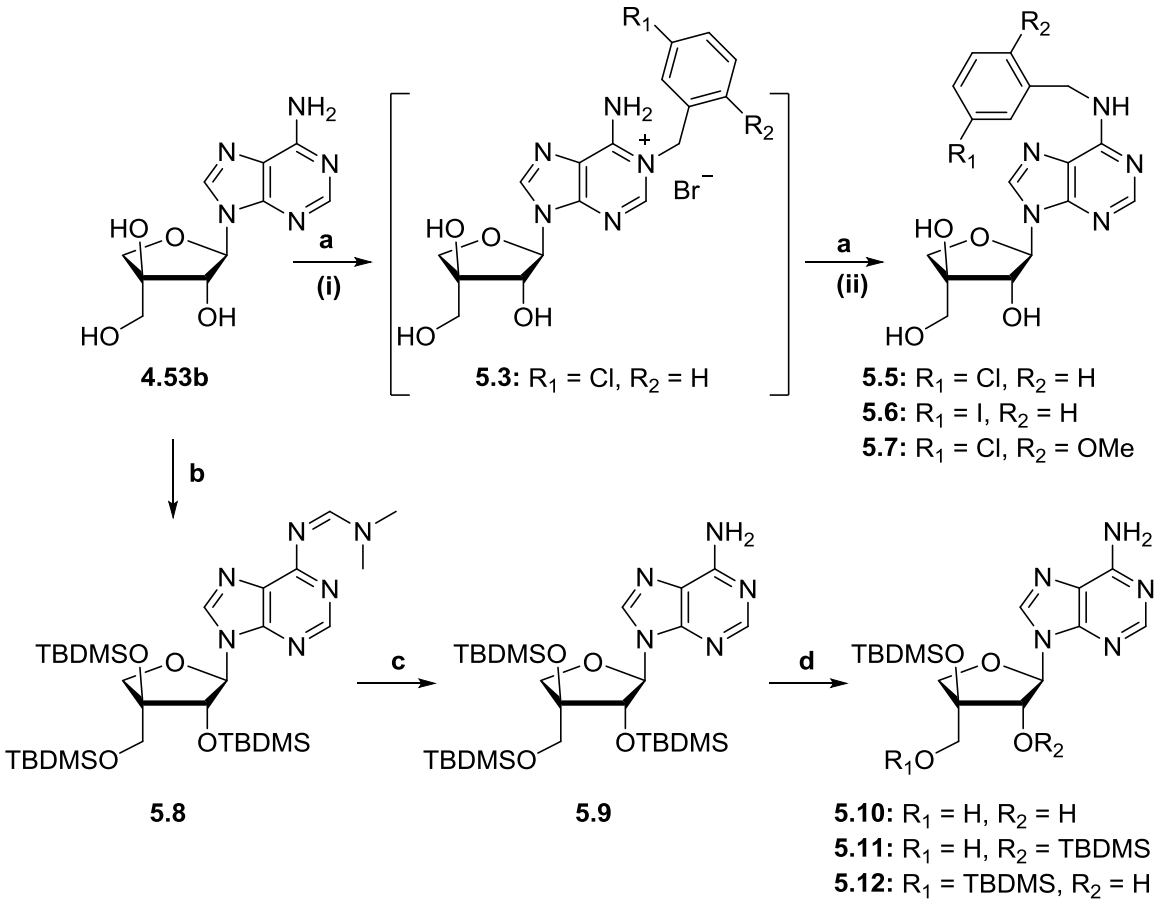
On the one hand, we envisaged to substitute the N^6 position of apioadenosine **4.3b** with substituted benzyl groups known to enhance A_3AR affinity (**5.24-26**, See Chapter 1, Section 1.4), on the other hand we planned to substitute the well-known ethylcarboxamide moiety for the 3′-CH_2OH group. However, synthetic problems in

introducing the ethylcarboxamide moiety motivated us to introduce an *N*-methyl (**5.30**) or N-ethylcarbamoyloxymethyl group instead.

5.2. Results and Discussion

5.2.1. Chemistry

5.2.1.1. Syntheses of α-D-apio-L-furanoadenosine derivatives



Scheme 5.1. Synthesis of N^6-substituted α-D-apio-L-furanoadenosines. *Reagents and conditions:* (a) (i) appropriate benzyl bromide, DMF, 50 °C, 48h; (ii) 25% NH_4OH, 50 °C, 24h or 90 °C, 3h, 20-56% over two steps; (b) TBDMSCl, imidazole, DMF, 100 °C, 3 days, 80%; (c) 7N NH_3 in MeOH, rt, 18h, 73%; (d) HF.pyridine, pyridine, THF, rt or TCA-H_2O, THF, rt, 20-30%.

The α-D-apio-L-furanoadenosines **4.53b** and its 3′-deoxy counterpart **4.52b** were prepared by microwave assisted synthesis as described in Chapter 4. Treatment of the former with the appropriate benzyl bromide first afforded the N^1-benzyl derivative, which was isolated in the case of the 3-chlorobenzyl derivative **5.3** (Scheme 5.1). Upon prolonged heating with aquous ammonia, the N^1-benzyl intermediate was converted to the desired N^6-benzylated compounds **5.5-5.7** via Dimroth rearrangement (Figure 5.2).[181] Although in many cases this method suffered from low yields, it allowed fast access to target molecules.

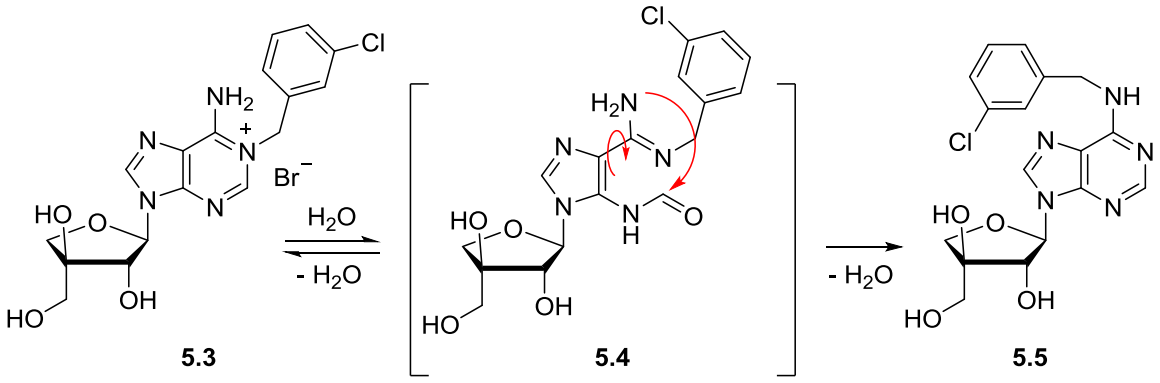
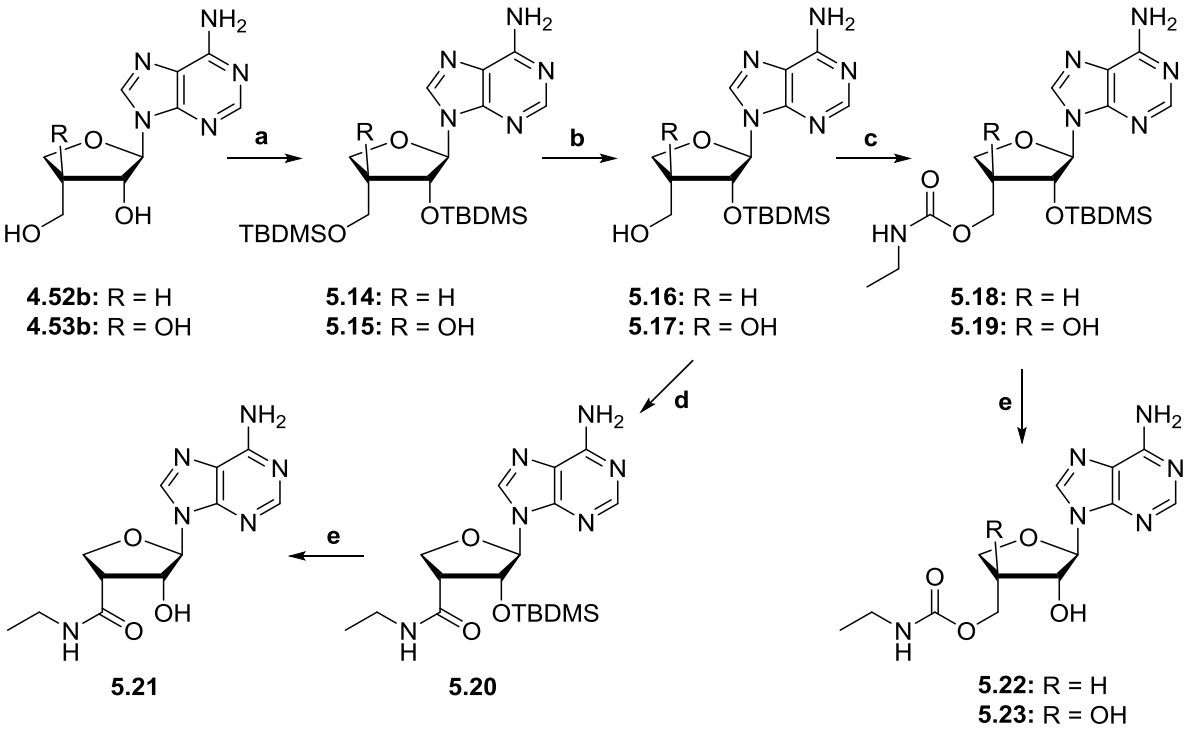


Figure 5.2. Mechanism of Dimroth rearrengment.

Selective modification of the 5′-position requires a suitable protecting strategy. First attempts were made to per-silylate the hydroxyl groups of compound **4.53b** (Scheme 5.1). Under mild conditions the tertiary hydroxyl group failed to react, while raising the temperature to 100 °C in DMF led to exclusive formation of the N^6-imide **5.8**. Since exploration of different solvents and scavenging bases did not allow the preparation of compound **5.9**, the latter was obtained by treatment of **5.8** with ammonia in MeOH.[182] Two different methods to selectively remove the TBDMS group from the primary hydroxyl of **5.9** resulted in complex reaction mixtures,[183,184] from which isomers **5.10** and **5.11** proved inseparable on TLC and by flash chromatography.

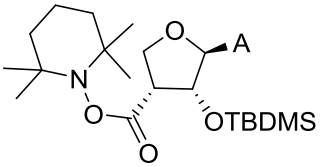
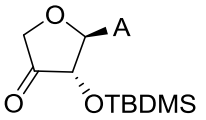
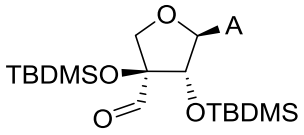


Scheme 5.2. Synthesis of sugar modified α-D-apio-L-furanoadenosines. *Reagents and conditions*: (a) TBDMSCl, imidazole, DMF, rt, 18h, 75-83%; (b) **5.14**, HF.pyridine, pyridine, THF, 0°C, 1h, rt, 1h, 82%; **5.15**, TCA-H_2O, THF, 0°C, 1h, rt, 1h, 87%; (c) (i) CDI, THF, rt, 3h; (ii) $EtNH_2$, rt, 16h, 55-95%; (d) (i) $RuCl_3$, $NaIO_4$, CH_3CN-CCl_4-H_2O, rt, 7h; (ii) CDI, THF, rt, 3h; (iii) $EtNH_2$, rt, 18h, 9% over three steps; (e) NH_4F, MeOH, 50 °C, 48h, 70-94%.

The aforementioned problems led us to synthesize the bis-silylated products **5.14** and **5.15**, which could be selectively deprotected to provide **5.16** and **5.17** in excellent yields (Scheme 5.2). Introduction of the desired 3′-carboxamide was tested on intermediate **5.16**. Conversion of the primary hydroxyl group of **5.16** to the corresponding carboxylic acid via a TEMPO-BAIB oxidation gave the corresponding TEMPO ester (Entry 1, Table 5.1).[185] This may be due to the high catalyst loading in the reaction, since slow conversion of the aldehyde intermediate forced us to add an extra amount of reagents. Efforts to convert the TEMPO-ester to the ethylamide failed. Under conditions of Jones oxidation (CrO_3 + dil. H_2SO_4) the starting material degraded. Oxidation using $RuCl_3$-$NaIO_4$,[186] followed by amide coupling, provided **5.20** and **5.18**, albeit in very low yields (Scheme 5.2). Removal of the TBDMS group in **5.20** gave the desired 3′-ethylcarboxamide **5.21**. Attempted ruthenium chloride

oxidation of **5.17** resulted in decarboxylation and further oxidation to the ketone as the only product (Entry 2, table 5.1). On the other hand, oxidation of **5.11** (as a mixture with **5.12**) did not proceed beyond the aldehyde stage, thus weakening the prospects of synthesizing the corresponding ethylcarboxamide via the carboxylic acid route. Sequential treatment of **5.16** and **5.17** with carbonyldiimidazole (CDI) and ethylamine produced 5′-*O*-ethylcarbamate derivatives **5.18** and **5.19**,[187] which were deprotected upon treatment with NH_4F in warm MeOH to give the desired 5′-*O*-ethylcarbamate apionucleosides **5.22** and **5.23** in excellent yields.

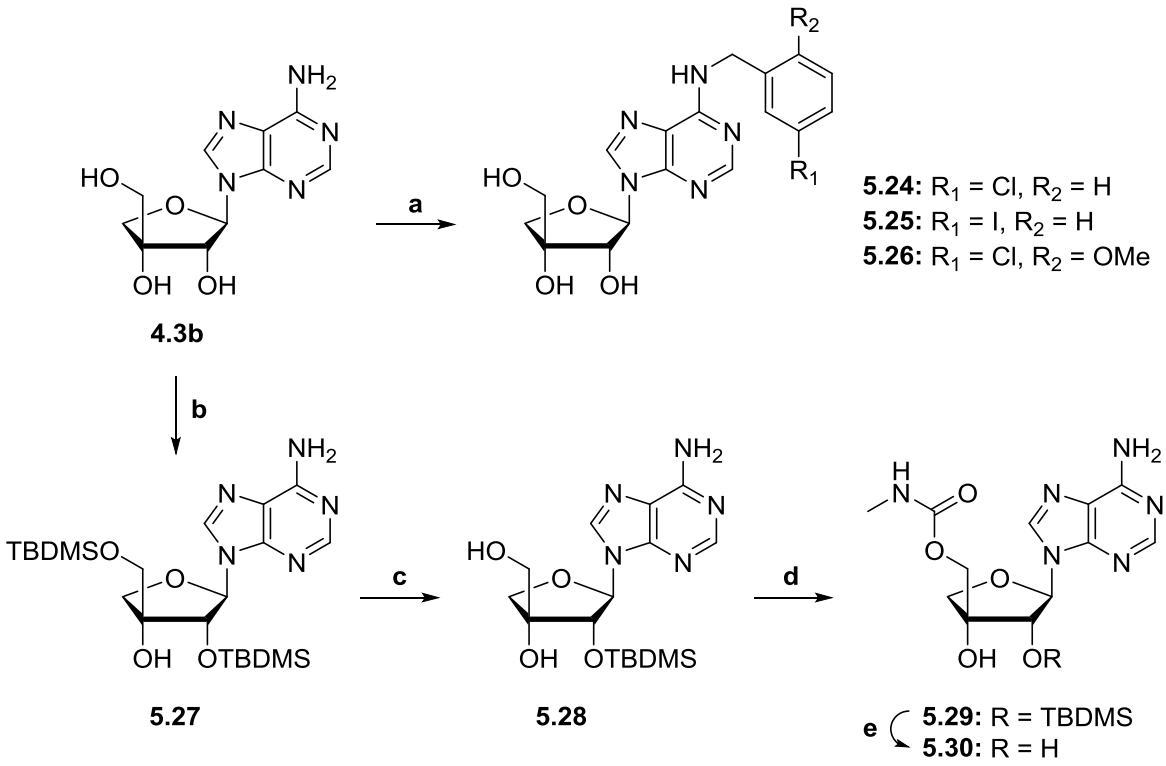
Table 5.1. Products from oxidation reactions

Entry	*Reactant*	*Condition*	*Product*	*HRMS (Obs)*
1	***5.16***	*TEMPO-BAIB, CH_3CN-H_2O, rt, 16h*		*$[M+H]^+$ - 519.3119*
2	***5.17***	*$RuCl_3$-$NaIO_4$, CH_3CN-CCl_4-H_2O, rt, 7h*		*$[M+H]^+$ - 350.1656* *$[M+H_3O]^+$ - 368.1762*
3	***5.11***	*$RuCl_3$-$NaIO_4$, CH_3CN-CCl_4-H_2O, 50 °C, 2days*		*$[M+H]^+$ - 494.2609* *$[M+H_3O]^+$ - 512.2742*

5.2.1.2. Syntheses of β-D-apio-D-furanoadenosine derivatives

Given the problems encountered for the oxidation of α-D-apio-L-furanoadenosine **5.17** and its conversion to carboxamide **5.21** and oxidation of **5.15**, the corresponding D-furano epimer was only converted to carbamate **5.30**. The synthesis of **4.3b** is described in chapter 4. The β-D-apio-D-furanoadenosine **4.3b** was benzylated at N^6 using similar reaction conditions as described for the L-furano counterpart (Scheme 5.3). RP-HPLC or PTLC was required to purify the target compounds, which were isolated in low yields. In the case of **5.26**, using excess of the benzyl bromide led to

degradation of the starting material and afforded bis-(2-methoxy-5-chlorobenzyl)adenine as observed by HRMS. By limiting the amount of this benzyl bromide to one equivalent, **5.26** could be obtained in 21% yield. The silylation and desilylation of **4.3b** rendered 2′-*O*-monosilylated species **5.28**, which upon treatment with carbonyldiimidazole and liq. methylamine gave **5.29**. The deprotection of TBDMS group furnished 5′-*O*-methylcarbamoyl -D-apio-D-furanoadenosine **5.30**.



Scheme 5.3. Synthesis of N^6-substituted and 5′-*O*-methylcarbamoyl β-D-apio-D-furanoadenosines. *Reagents and conditions:* (a) (i) appropriate benzyl bromide, DMF, rt, 48h; (ii) 25% NH_4OH, 50 °C, 24h, 8-21% over two steps; (b) TBDMSCl, imidazole, DMF, rt, 18h, 74%; (c) TCA-H_2O, THF, 0°C, 1h, rt, 3h, 35%; (c) (i) CDI, THF, rt, 3h; (ii) $MeNH_2$, rt, 16h, 72%; (e) NH_4F, MeOH, 50 °C, 48h, 90%.

5.2.2. Pharmacological evaluation

For the apio-type adenosine derivatives prepared in this study (**5.3**, **5.5-7**, **5.21-26** and **5.30**) we measured the binding affinities at the hA_1, hA_{2A} and hA_3AR. The results are reported in Table 5.2. The ability of each of these adenosine derivatives to compete for radioligand binding at each of these hARs was evaluated at a fixed concentration

of 10 μM and a full inhibition curve was determined for compound **5.7** and **5.26** at the A_3AR (Figure 5.3). Most compounds only caused marginal displacement of the radioligands from all AR subtypes tested. The N^6-benzyl derivatives **5.5-7**, **5.24-26** and **5.30** showed weak affinity at A_3AR. However, N^6-(5-chloro-2-methoxybenzyl)-α-D-apio-L-furanoadenosine **5.7** showed a binding affinity to the A_3AR in the low micromolar range (K_i: 3 ± 0.75 μM) and behaved as a partial agonist. Surprisingly, the D-furano counterpart **5.26** displayed similar binding affinity and a functional data suggest that it behaves as an antagonist. Comparison with the A_3AR binding affinity of N^6-(5-chloro-2-ethoxy)benzyladenosine (K_i: 1.31 nM), the structural isomer from which **5.7** was derived, indicates that substitution of the apiofuranose for a ribofuranose moiety is detrimental for binding to the ARs.

Table 5.2. Affinities of apioadenosine derivatives.[a]

Compound number	*% inhibition or K_i (nM)*		
	hA₁	***hA₂A***	***hA₃***
5.3	*6 ± 5%*	*12 ± 7%*	*3 ± 2%*
5.5	*19 ± 1%*	*15 ± 5%*	*44 ± 1%*
5.6	*35 ± 5%*	*22 ± 2%*	*45 ± 1%*
5.7	*13 ± 5%*	*14 ± 5%*	*3070 ± 750*
5.21	*8 ± 7%*	*9 ± 5%*	*10 ± 2%*
5.22	*9 ± 5%*	*12 ± 4%*	*6 ± 2%*
5.23	*7 ± 2%*	*2 ± 2%*	*8 ± 1%*
5.24	*10 ± 6%*	*10 ± 8%*	*43 ± 5%*
5.25	*27 ± 9%*	*17 ± 11%*	*48 ± 6%*
5.26	*12 ± 3%*	*17 ± 6%*	*978 ± 150*
5.30	*12 ± 4%*	*10 ± 8%*	*26 ± 6 %*

[a] Binding in membranes of CHO or HEK293 (A_{2A} only) cells stably expressing one of three *h*AR subtypes. Percent refers to inhibition of binding at 10 μM. The binding affinity for *h*A_1, A_{2A}, and A_3ARs was expressed as K_i values using agonists [^{3}H]N^6-R-phenylisopropyladenosine, [^{3}H]2-[*p*-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine, or [^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide, respectively.

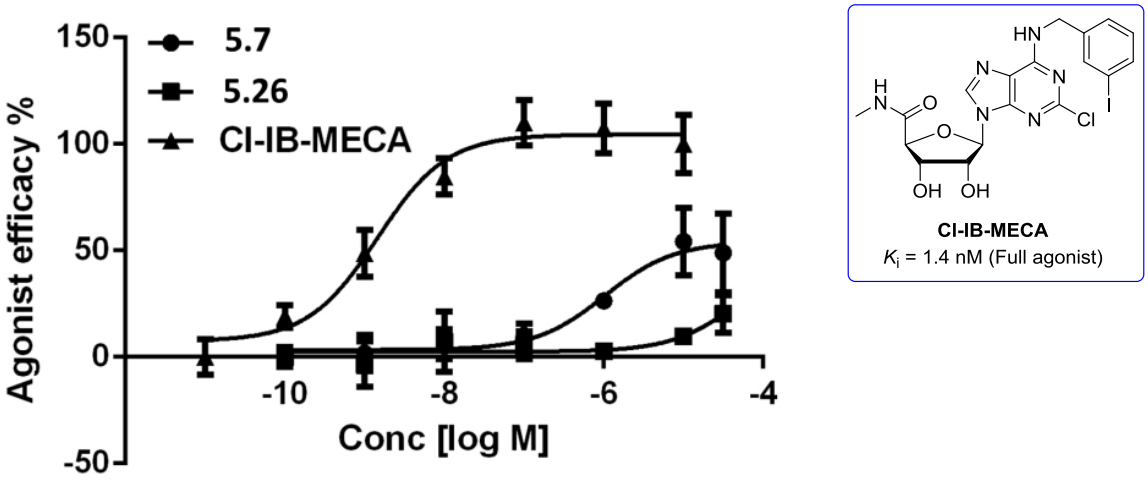


Figure 5.3. Efficacy curve for compound **5.7** and **5.26**.

5.2.3. Homology modelling studies

Both compounds **5.7** and **5.26** show docking poses at the hA_3AR well superimposable to the binding mode of Cl-IB-MECA (for structure of Cl-IB-MECA, see Figure 5.3). For all nucleosides featuring N^6-benzyl adenosine type structure, the interactions are predictably similar. The side chain of Asn250 (6.55, using Ballesteros-Weinstein notation[188]) in the hA_3AR homology model strongly interacts through two H-bonds involving the 6-amino group and the N^7 atom of the adenine ring (Figure 5.4). Moreover, the adenine ring is anchored inside the binding site by a π–π stacking interaction with Phe168 (EL2) and strong hydrophobic contacts with Leu246 (6.51) and Ile268 (7.39). The N^6-3-iodobenzyl ring is accommodated in a hydrophobic pocket delimited by TM5, TM6 and EL2 to form strong hydrophobic interactions with Val169 (EL2), Met174 (5.35) and Ile253 (6.58). Finally, the 3′- and 2′-hydroxyl groups of the ribose ring form H-bonds with Ser271 (7.42) and His272 (7.43), respectively, while the 5'-N-methyluronamido moiety forms a H-bond with Thr94 (3.36). These latter interactions involving the ribose moiety are particular to agonist binding, as shown by the comparison of the reported crystallographic structures of the $hA_{2A}AR$ in complex with agonists and antagonists,[132] and are supposed to be important for the activation process in the AR family.

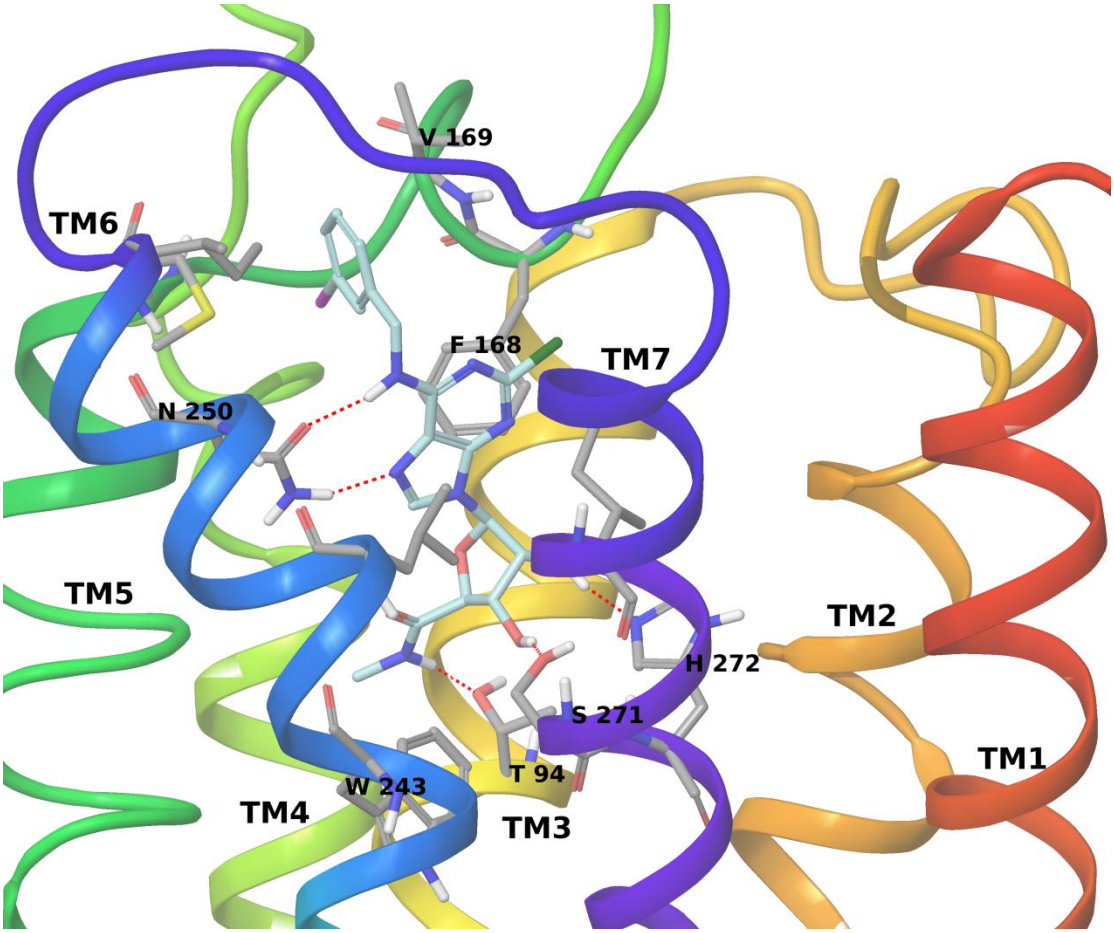


Figure 5.4. Binding mode of Cl-IB-MECA with A_3 AR.

It has been previously shown that the 4'-truncation of nucleoside derivatives that are potent A_3AR ligands leads to compounds with good affinity for the A_3 receptor but with decreased efficacy, i.e. behaving as partial agonist or antagonist of this subtype. Docking of nucleoside analogues in the hA_3AR homology model indicated that the absence of a hydroxymethyl or uronamide substituent at the 4'-position prevented these compounds from interacting with Thr94 (3.36). This residue is a conserved recognition point in agonist binding, and therefore the lack of this interaction was considered as a reason for the low efficacy profile of 4'-truncated nucleosides.[189]

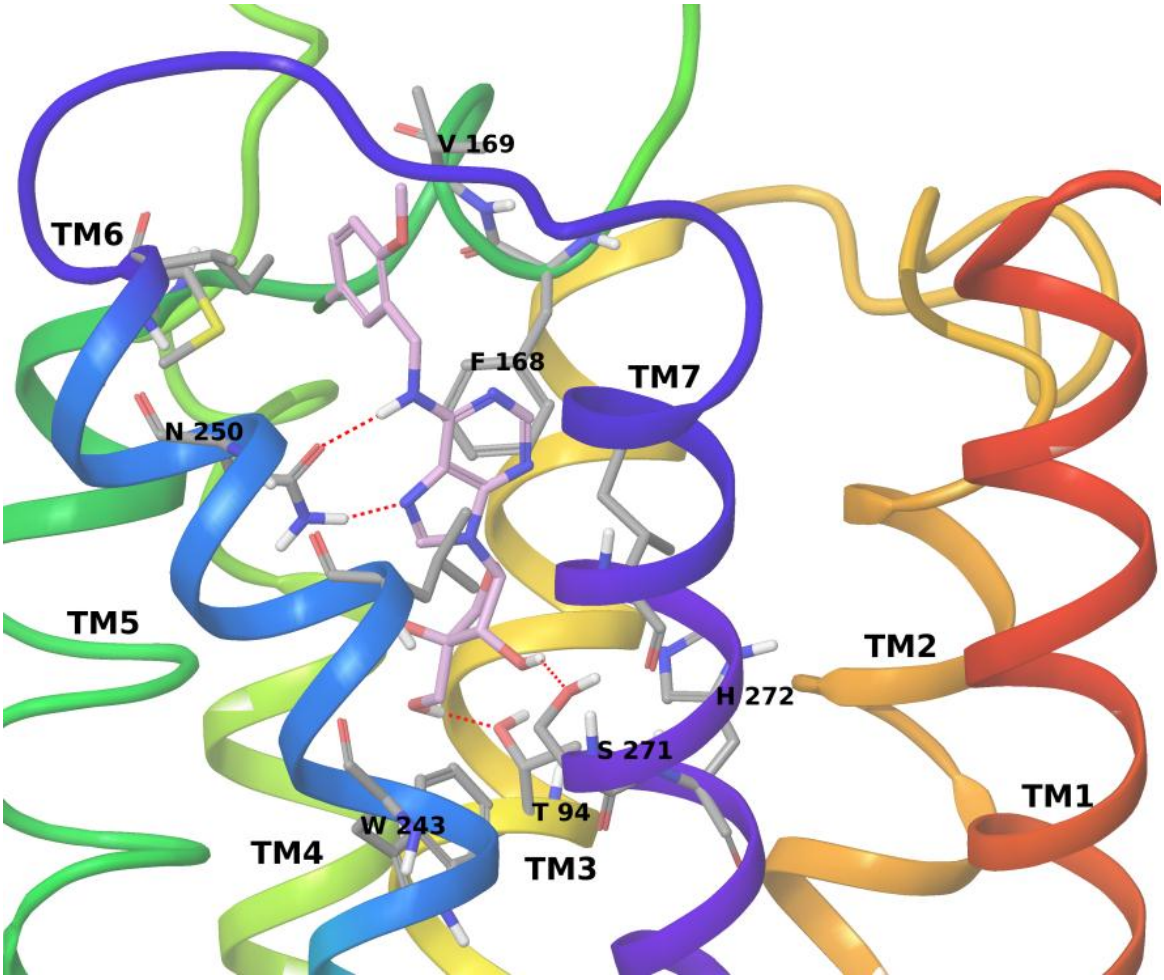


Figure 5.5. Important interactions for compound **5.7** with A_3 AR.

For compounds **5.7** and **5.26** conserved interactions stabilizing the adenine core and the N^6 substituent are similar to Cl-IB-MECA at the hA_3AR. Differences can be observed in the interactions formed by the sugar moiety; in fact, both compounds form only two of the three H-bonds predicted for binding of the full agonist Cl-IB-MECA. In particular, compound **5.7** forms H-bonds with Thr94 (3.36) and Ser271 (7.42) and not with His272 (7.43) (Figure 5.5), while compound **5.26** forms two H-bonds with Ser271 (7.42) and His272 (7.43), but it cannot reach Thr94 in TM3 (Figure 5.6). Therefore, compound **5.26** show a binding mode similar to the one observed for other 4'-truncated nucleosides and the missing interaction with Thr94 (3.36) is consistent with its lack of receptor activation, indicative of antagonist behavior. However, the ability of partial agonist **5.7** to bridging between TM3 and TM7 likely correlates with

the ability to induce the conformational changes required for receptor activation, such as an inward movement of TM7. Thus, the preferred modeled binding modes of **5.7** and **5.26** differ in the crucial interaction with Thr94, which seems to be associated with residual efficacy at the hA_3AR in apionucleosides.

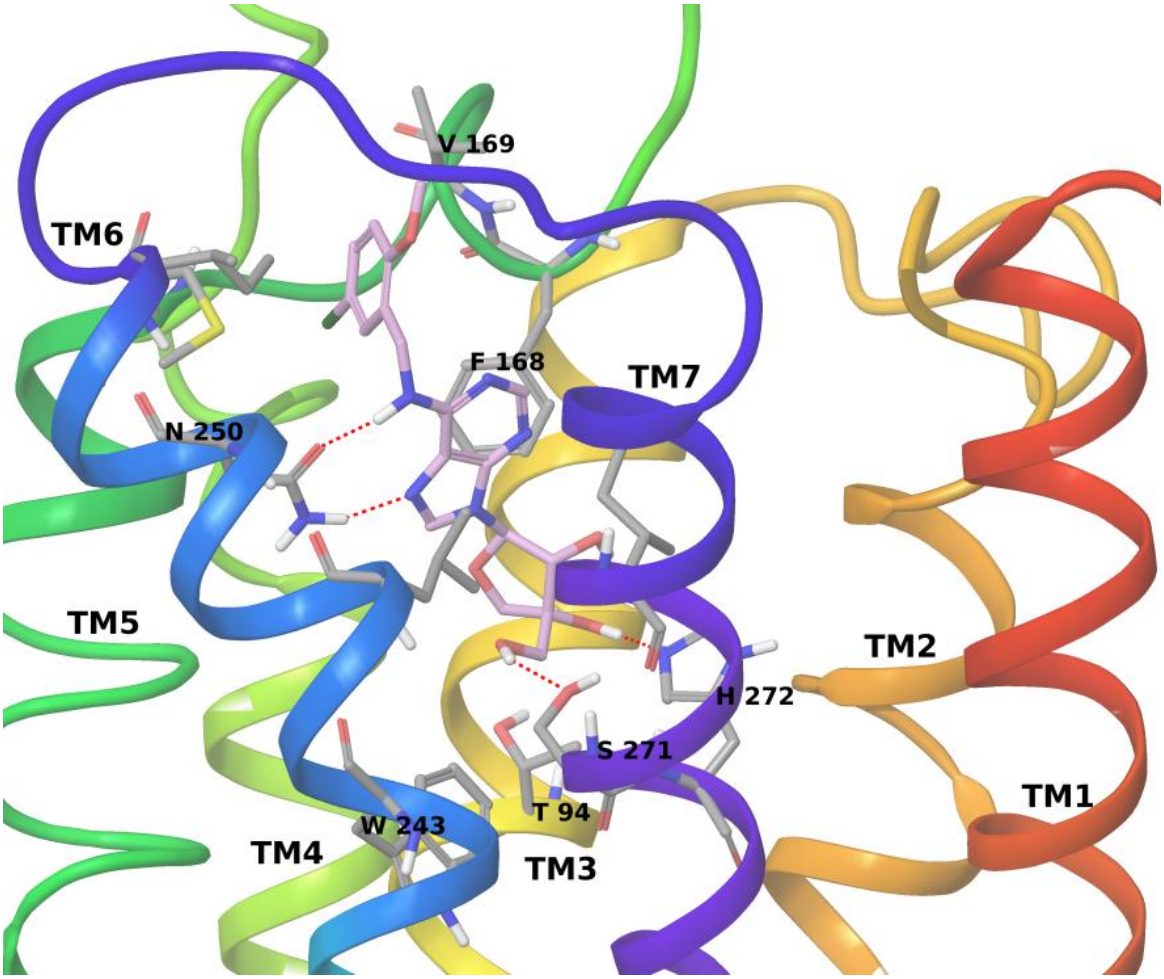


Figure 5.6. Important interactions for compound **5.26** with A_3 AR.

5.3. Conclusions

In summary, we have synthesized a small number of modified apioadenosines as potential A_3AR ligands. Generally, substituting an apiofuranose for a ribofuranose moiety is detrimental for binding to the ARs. Nevertheless, selected N^6-substituted 9-

(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenines showed weak but selective binding affinity for the A_3AR. Remarkably, this was also the case for their 3′-epimers (D-apio-L-furanosyl analogues), which are capable of partially activating the A_3AR.

5.4. Experimental Section

5.4.1. Synthesis

All reagents were from standard commercial sources and of analytic grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. Moisture sensitive reactions were carried out under argon atmosphere. A temperature of 25±5 °C is referred to as 'room temperature/ rt'. Precoated Merck silica gel F254 plates were used for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (200-400 mesh, 60 Å, Biosolve, Valkenswaard, The Netherlands). RP-HPLC was performed using Waters XBridge OBD^{TM} Prep C18 5µm column @ 17.5 mL/min flow rate. NMR spectra were determined using a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals or TMS as internal standard. Exact mass measurements were performed on a Waters LCT PremierXETMTime of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1) mixture at 10 µL/min. For nucleosides, NMR signals of sugar protons and carbons are indicated with a prime, and signals of base protons and carbons are given without a prime.

1′-[*N*^{1}-(3-Chlorobenzyl)-adenin-9-yl]-α-D-apio-L-furanose bromide (5.3): To a solution of apioadenosine **4.53b** (25 mg, 0.094mmol) in anh. DMF (1 mL) was added 3-chlorobenzyl bromide (50µL, 0.374 mmol) and the mixture was stirred at 50 °C for 48h. The solvent was evaporated under reduced pressure and the residue was purified first by column chromatography (8-12% MeOH in CH_2Cl_2) and then with preparative thin layer chromatography (15% MeOH in CH_2Cl_2, R_f: 0.2) to afford the title

compound **5.3** as a white solid (16 mg, 44%). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.75 (d, *J* = 11.72 Hz, 1H, 5′-H), 3.85 (d, *J* = 11.72 Hz, 1H, 5′-H), 4.18 (d, *J* = 9.67 Hz, 1H, 4′-H), 4.25 (d, *J* = 9.37 Hz, 1H, 4′-H), 4.42 (d, *J* = 0.88 Hz,1H, 2′-H), 5.63 (s, 2H, ArC*H*$_2$), 6.15 (d, *J* = 1.46 Hz, 1H, 1′-H), 7.14 - 7.24 (m, 1H, Ar-H), 7.33 - 7.46 (m, 3H, Ar-H), 8.55 (s, 1H, 8-H), 8.68 (s, 1H, 2-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 53.40 (Ar*C*H$_2$), 63.43 (5′-C), 78.14 (4′-C), 81.97 (2′-C), 82.77 (3′-C), 94.30 (1′-C), 120.91 (5-C), 126.28, 128.22 (Ar 2,6-Cs), 130.06, 131.98 (Ar 3,4-Cs), 136.26 (Ar 5-C), 136.52 (Ar 1-C), 144.79 (8-C), 148.09 (4-C), 148.42 (2-C), 152.29 (6-C). ESI-HRMS for $[C_{17}H_{18}ClN_5O_4 + H]^+$ calcd, 392.1126; found, 392.1139.

1′-[*N*^{6}-(3-Chlorobenzyl)-adenin-9-yl]-α-D-apio-L-furanose (5.5): To a solution of apioadenosine **4.53b** (25 mg, 0.094mmol) in anh. DMF (1 mL) was added 3-chlorobenzyl bromide (50µL, 0.374 mmol) and the mixture was stirred at 50 °C for 48h. Ammonium hydroxide (25%, 3.0 mL) was added and stirred at 50 °C for 24h (alternatively, at 90 °C for 3h was used only for **5.5**). The volatiles were evaporated under reduced pressure and the residue purified by column chromatography (3-6% MeOH in CH_2Cl_2) to afford desired product **5.5** (20 mg, 56%) as a white solid. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ ppm 3.62 (d, *J* = 5.56 Hz, 2H, 5′-H), 3.99 (d, *J* = 9.08 Hz, 1H, 4′-H), 4.05 (d, *J* = 9.08 Hz, 1H, 4′-H), 4.40 (dd, *J* = 4.98, 2.93 Hz, 1H, 2′-H), 4.64 (t, *J* = 5.56 Hz, 1H, 5′-OH), 4.71 (br.s, 2H, Ar-C*H*$_2$), 5.32 (s, 1H, 3′-OH), 5.86 (d, *J* = 5.27 Hz, 1H, 2′-OH), 5.92 (d, *J* = 2.93 Hz, 1H, 1′-H), 7.23 - 7.37 (m, 3H, Ar-H's), 7.39 (s, 1H, Ar-H), 8.23 (s, 1H, 2-H), 8.35 (s, 1H, 8-H), 8.44 (br.s, 1H, NH). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 44.68 (Ar*C*H$_2$), 63.74 (5′-C), 77.69 (4′-C), 82.33 (2′-C), 82.73 (3′-C), 94.27 (1′-C), 120.82 (5-C), 126.96, 128.34, 128.56, 131.16 (Ar 2,3,4,6-C's), 135.50 (Ar 5-C), 141.44 (8-C), 143.08 (Ar 1-C), 149.50 (4-C), 153.79 (2-C), 156.08 (6-C).ESI-HRMS for $[C_{17}H_{18}ClN_5O_4 + H]^+$ calcd, 392.1126; found, 392.1105.

1′-[*N*^{6}-(3-Iodobenzyl)-adenin-9-yl]-α-D-apio-L-furanose (5.6): Following the protocol for the synthesis of compound **5.5**, the title compound (33 mg, 36%) was obtained from apioadenosine **4.53b** (50 mg, 0.19 mmol) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.76 (d, *J* = 11.57 Hz, 1H, 5′-H), 3.84 (d, *J* = 11.57 Hz, 1H, 5′-H), 4.12 (d, *J* = 9.45 Hz, 1H, 4′-H), 4.18 (d, *J* = 9.45 Hz, 1H, 4′-H), 4.37 (d, *J* =

1.46 Hz, 1H, 2′-H), 4.78 (br.s, 2H, ArCH_2), 6.02 (d, *J* = 1.75 Hz, 1H, 1′-H), 7.07 (t, *J* = 7.79 Hz, 1H, Ar 5-H), 7.37 (d, *J* = 7.66 Hz, 1H, Ar 2-H), 7.58 (d, *J* = 7.87 Hz, 1H, Ar 4-H), 7.74 (s, 1H, Ar 6-H), 8.26 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 44.45 (Ar*C*H_2), 63.60 (5′-C), 77.54 (4′-C), 82.19 (2′-C), 82.59 (3′-C), 94.13 (1′-C), 94.96 (Ar 5-C), 120.68 (5-C), 127.84(Ar 2-C), 131.37 (Ar 3-C), 137.33 (Ar 4-C), 137.51 (Ar 6-C), 141.30 (8-C), 143.11 (Ar 1-C), 149.46 (4-C), 153.66 (2-C), 155.91 (6-C).ESI-HRMS for $[C_{17}H_{18}IN_5O_4 + H]^+$ calcd, 484.0482; found, 484.0470.

1′-[*N*^{6}-(2-Methoxy-5-chlorobenzyl)-adenin-9-yl]-α-D-apio-L-furanose (5.7): Following the protocol used for the synthesis of compound **5.5**, apioadenosine **4.53b** (50 mg, 0.19 mmol) was converted to the title compound **5.7** (15 mg, 20%), which was obtained as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.75 (d, *J* = 11.4 Hz, 1H, 5′-H), 3.84 (d, *J* = 11.7 Hz, 1H, 5′-H), 3.87 (s, 3H, Ar-OCH_3), 4.12 (d, *J* = 9.3 Hz, 1H, 4′-H), 4.18 (d, *J* = 9.9 Hz, 1H, 4′-H), 4.37 (d, *J* = 1.2 Hz, 1H, 2′-H), 4.77 (br.s, 2H, ArCH_2), 6.02 (d, *J* = 1.5 Hz, 1H, 1′-H), 6.94 (d, *J* = 8.7 Hz, 1H, Ar 5-H), 7.21 (dd, *J* = 8.7, 2.7 Hz, 1H, Ar 4-H), 7.25 (d, *J* = 2.4 Hz, 1H, Ar 6-H), 8.26 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 40.30 (Ar-*C*H_2), 56.27 (ArO*C*H_3), 63.60 (5′-C), 77.56 (4′-C), 82.17 (3′-C), 82.60 (2′-C), 94.15 (1′-H), 112.83 (Ar 3-C), 120.66 (5-C), 126.30 (Ar 5-C), 129.07 (Ar 6-C), 129.09 (Ar 4-C), 130.00 (Ar 1-C), 141.24 (8-C), 149.20 (4-C), 153.68 (2-C), 155.99 (6-C), 157.57 (Ar 2-C). ESI-HRMS for $[C_{18}H_{20}ClN_5O_5 + H]^+$ calcd, 422.1231; found, 422.1236.

1′-[*N*^{6}-(*N*,*N*-Dimethylformamidine)-adenin-9-yl]-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.8): To a solution of apioadenosine **4.53b** (150 mg, 0.56 mmol) in anh. DMF (5 mL) was added imidazole (458 mg, 6.73 mmol) and TBDMSCl (845 mg, 5.6 mmol). The mixture was heated to 100 °C for 3 days, cooled and a saturated aqueous NH_4Cl solution was added. The product was extracted with EtOAc (3 X 20 mL) and the combined organic layers were dried over anh. Na_2SO_4. The residue obtained after evaporation of the organic layers was subjected to column chromatography (15-30% EtOAc in hexanes) to afford **5.8** (300 mg, 80 %) as a colorless glassy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.38 (s, 3H, $SiCH_3$), -

0.07 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$), 0.18 (s, 3H, $SiCH_3$), 0.78 (s, 9H, $C(CH_3)_3$), 0.93 (s, 9H, $C(CH_3)_3$), 0.96 (s, 9H, $C(CH_3)_3$), 3.21 (s, 3H, NCH_3), 3.27 (s, 3H, NCH_3), 3.71 (d, *J* = 10.54 Hz, 1H, 4′-H), 3.95 (d, *J* = 10.54 Hz, 1H, 4′-H), 4.25 (d, *J* = 8.49 Hz, 1H, 5′-H), 4.34 (d, *J* = 8.79 Hz, 1H, 5′-H), 5.16 (d, *J* = 6.15 Hz, 1H, 2′-H), 5.75 (d, *J* = 6.15 Hz, 1H, 1′-H), 7.94 (s, 1H, 8-H), 8.54 (s, 1H, 2-H), 8.97 (s, 1H, N^6CH). ESI-HRMS for $[C_{31}H_{60}N_6O_4Si_3 + H]^+$ calcd, 665.4062; found, 665.4075.

1′-(Adenin-9-yl)-2′,3′,5′-*O*-tri(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.9): The N^6-dimethyformamidine derivative **5.8** (300 mg, 0.45 mmol) was dissolved in a 7N solution of ammonia in MeOH and stirred at room temperature for 18h. The mixture was evaporated and the residue purified by column chromatography (20-40% EtOAc in hexanes) to afford **5.9** (200 mg, 73%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.34, -0.04, 0.09, 0.14, 0.15, 0.17 (s, 6x3H, $SiCH_3$), 0.79 (s, 9H, $C(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 0.97 (s, 9H, $C(CH_3)_3$), 3.71 (d, *J* = 10.54 Hz, 1H, 4′-H), 3.96 (d, *J* = 10.84 Hz, 1H, 4′-H), 4.25 (d, *J* = 8.79 Hz, 1H, 5′-H), 4.32 (d, *J* = 8.49 Hz, 1H, 5′-H), 5.13 (d, *J* = 6.15 Hz, 1H, 2′-H), 5.61 (s, 2H, NH_2), 5.73 (d, *J* = 6.15 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H) 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, CDCl3) δ ppm -5.29, -5.27, -5.19, -4.65, -2.85, -2.70 ($SiCH_3$), 17.97, 18.41, 18.75 ($C(CH_3)_3$), 25.66, 26.04, 26.19 ($C(CH_3)_3$), 64.94 (5′-C), 74.45 (4′-C), 83.16 (2′ & 3′-C), 90.42 (1′-C), 120.57 (5-C), 140.12 (8-C), 150.07 (4-C), 153.22 (2-C), 155.55 (6-C). ESI-HRMS for $[C_{28}H_{55}N_5O_4Si_3 + H]^+$ calcd, 610.3640; found, 610.3651.

Method A: A solution of compound **5.9** (180 mg, 0.3 mmol) in anh. THF (4.25 mL) was placed in a Teflon flask under inert atmosphere and cooled to 0 °C. In a separate polypropylene flask, anh. THF (1.25 mL) and anh. pyridine (0.5 mL) were placed under inert atmosphere and cooled to 0 °C. A solution of 70% HF in pyridine (0.5 mL) was added dropwise to the latter mixture. 1.7 mL of the resulting chilled THF-pyridine–HF.pyridine (2.5:1:1) mixture was added dropwise to the solution of **5.9** and the mixture was stirred at 0 °C for 1h. The ice-bath was removed and reaction continued at room temperature for 24h. The reaction mixture was poured to an ice-cold solution of $NaHCO_3$ (1.5 g in 8 mL) with vigorous stirring. The product was

extracted with EtOAc (3 X 20 mL), washed with brine and dried over anh. Na_2SO_4. The residue obtained after evaporation was subjected to flash column chromatography (2–5% MeOH in CH_2Cl_2) to obtain following compounds as white foam. Starting material **5.9** (70 mg, 39%), compound **5.10** (33 mg, 29%) and mixture of compounds **5.11** and **5.12** (~2:1, 37 mg, 25%).

Method B: To a cooled (0 °C) solution of compound **5.9** (70 mg, 0.12 mmol) in anh. THF (1 mL) was added a solution of trichloroacetic acid (507 mg, 3.1 mmol) in water (0.27 mL) and the mixture was stirred for 20 min at 0 °C, and for 24h at room temperature. The reaction mixture was cooled and neutralized with a cold sat. $NaHCO_3$ solution. The products were extracted with EtOAc (3 X 30 mL), the combined organic phase was dried over anh.Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography to afford following compounds: Starting material **5.9** (20 mg, 29%), compound **5.10** (10 mg, 23%) and a mixture of compounds **5.11** and **5.12** (~2:1, 17 mg, 30%).

1′-(Adenin-9-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.10): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.08 (s, 6H, $(SiCH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 3.74 (d, *J* = 10.54 Hz, 1H, 4′-H), 3.84 (d, *J* = 10.25 Hz, 1H, 4′-H), 3.99 (d, *J* = 9.08 Hz, 1H, 5′-H), 4.06 (d, *J* = 8.79 Hz, 1H, 5′-H), 4.41 (dd, *J* = 5.27, 3.22 Hz, 1H, 2′-H), 5.32 (s, 1H, 5′-OH), 5.88 (d, *J* = 5.56 Hz, 1H, 2′-OH), 5.90 (d, *J* = 3.22 Hz, 1H, 1′-H), 7.26 (br. s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.30 (s, 1H, 8-H). ^{1}H NMR (75 MHz, DMSO-d_6) δ ppm -5.48, -5.37 ($SiCH_3$), 18.05 ($C(CH_3)_3$), 25.79 ($C(CH_3)_3$), 64.25 (5′-C), 75.07 (4′-C), 79.93 (2′-C), 80.13 (3′-C), 90.57 (1′-C), 118.76 (5-C), 139.62 (8-C), 148.99 (4-C), 152.39 (2-C), 155.90 (6-C). ESI-HRMS for $[C_{16}H_{27}N_5O_4Si + H]^+$ calcd, 382.1911; found, 382.1903.

1′-(Adenin-9-yl)-2′,3′-*O*-di(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.11): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm -0.18, -0.05, 0.08 (s, 4X3H, $SiCH_3$), 0.78, 0.90 (s, 2X9H, $C(CH_3)_3$), 3.67 (d, *J* = 10.25 Hz, 1H, 5′-H), 3.82 (d, *J* = 10.25 Hz, 1H, 5′-H), 4.01 (d, *J* = 8.79 Hz, 1H, 4′-H), 4.11 (d, *J* = 8.79 Hz, 1H, 4′-H), 4.68 (d, *J* = 4.39 Hz, 1H, 2′-H), 5.33 (s, 1H, 5′-OH), 5.89 (d, *J* = 4.39 Hz, 1H, 1′-H), 7.27 (br. s, 2H, NH_2),

8.15 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2775.

1′-(Adenin-9-yl)-3′,5′-*O*-di(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.12): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.10, 0.10, 0.12, 0.13 (s, 4X3H, $SiCH_3$), 0.87, 0.92 (s, 2X6H, $C(CH_3)_3$), 3.75 (d, *J* = 10.84 Hz, 1H, 5′-H), 3.87 (d, *J* = 11.13 Hz, 1H, 5′-H), 4.06 (d, *J* = 8.20 Hz, 1H, 4′-H), 4.19 (d, *J* = 8.20 Hz, 1H, 4′-H), 5.00 (t, *J* = 5.13 Hz, 1H, 2′-H), 5.76 (d, *J* = 5.86 Hz, 1H, 1′-H), 5.84 (d, *J* = 4.98 Hz, 1H, 2′-OH), 7.27 (br. s, 2H, NH_2), 8.12 (s, 1H, 2-H), 8.24 (s, 1H, 8-H). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2775.

1′-(Adenin-9-yl)-2′,5′-*O*-di(*tert*-butyldimethylsilyl)-3′-deoxy-α-D-apio-L-furanose (5.14): To a solution of 3′-deoxyapioadenosine **4.52b** (170 mg, 0.67 mmol) in anh. DMF (2 mL) was added imidazole (230 mg, 3.38 mmol), followed by TBDMSCl (305 mg, 2.03 mmol) and the mixture was stirred at room temperature for 18h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 X 20 mL). The combined organic layer was dried over anh. Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using 3-5% MeOH in CH_2Cl_2 to afford **5.14** (242 mg, 75%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.05 (s, 6H, $Si(CH_3)_2$), 0.07 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.88 (s, 9H, $C(CH_3)_3$), 0.90 (s, 9H, $C(CH_3)_3$), 2.56 (qt, *J* = 7.81, 5.71 Hz, 1H, 3′-H), 3.75 (dd, *J* = 9.96, 7.91 Hz, 1H, 5′-H), 3.87 (dd, *J* = 10.10, 6.00 Hz, 1H, 5′-H), 4.08 (t, *J* = 8.35 Hz, 1H, 4′-H), 4.45 (dd, *J* = 8.20, 7.62 Hz, 1H, 4′-H), 4.85 (dd, *J* = 4.98, 2.05 Hz, 1H, 2′-H), 5.55 (br.s, 2H, NH_2), 5.90 (d, *J* = 2.05 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.47, -5.39, -5.28, -4.70 ($SiCH_3$), 17.99, 18.26 ($C(CH_3)_3$), 25.69, 25.87 ($C(CH_3)_3$), 44.77 (3′-C), 60.02 (5′-C), 71.91 (4′-C), 76.02 (2′-C), 92.47 (1′-C), 120.45 (5-C), 138.77 (8-C), 149.35 (4-C), 152.95 (2-C), 155.24 (6-C). ESI-HRMS for $[C_{22}H_{41}N_5O_3Si_2]$ calcd, 480.2826; found, 480.2828.

1′-(Adenin-9-yl)-2′,5′-*O*-di(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.15): To a solution of Apioadenosine **4.53b** (150 mg, 0.56 mmol) in anh. DMF (2 mL) was added imidazole (155 mg, 2.28 mmol), followed by TBDMSCl (253 mg, 1.68 mmol) and the mixture was stirred at room temperature for 18h. The reaction mixture was

diluted with water (10 mL) and extracted with EtOAc (3 X 20 mL). The combined organic layer was dried over anh. Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using 3-5% MeOH in CH_2Cl_2 to afford **5.15** (230 mg, 83%) as a white foam. ^{1}H NMR (300 MHz, CDCl3) δ ppm 0.08 (s, 3H, $SiCH_3$), 0.10 (s, 6H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.91 (s, 18H, $C(CH_3)_3$), 3.75 (d, *J* = 9.96 Hz, 1H, 4′-H), 3.92 (d, *J* = 10.25 Hz, 1H, 4′-H), 4.08 (d, *J* = 9.08 Hz, 1H, 5′-H), 4.21 (d, *J* = 9.37 Hz, 1H, 5′-H), 4.45 (d, *J* = 1.46 Hz, 1H, 2′-H), 5.80 (br.s, 2H, NH), 5.95 (d, *J* = 1.76 Hz, 1H, 1′-H), 8.13 (s, 1H, 8-H) 8.32 (s, 1H, 2-H). ^{13}C NMR (75 MHz, CDCl3) δ ppm -5.51, -5.31, -5.22, -4.55 ($SiCH_3$), 17.87, 18.37 ($C(CH_3)_3$), 25.66, 25.91 ($C(CH_3)_3$), 63.15 (4′-C), 76.68 (5′-C), 81.43 (3′-C), 81.53 (2′-C), 92.75 (1′-C), 119.81 (5-C), 140.04 (8-C), 149.27 (4-C), 152.68 (2-C), 155.31 (6-C). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2783.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-α-D-apio-L-furanose (5.16): Following the desilylation method A (which was slightly modified in that the reaction mixture was stirred for 1h at room temperature) starting material **5.14** (240 mg, 0.5 mmol) was converted to **5.16** (150 mg, 82%), obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.91 (s, 9H, $C(CH_3)_3$), 2.14 (br. s, 1H, 5′-OH), 2.66 (tq, *J* = 7.94, 5.74 Hz, 1H, 3′-H), 3.89 (d, *J* = 5.57 Hz, 2H, 5′-H), 4.19 (t, *J* = 8.35 Hz, 1H, 4′-H), 4.46 (dd, *J* = 8.49, 7.91 Hz, 1H, 4′-H), 5.07 (dd, *J* = 5.71, 2.49 Hz, 1H, 2′-H), 5.56 (br. s, 2H, NH_2), 5.89 (d, *J* = 2.34 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.22, -4.72 ($SiCH_3$), 17.94 ($C(CH_3)_3$), 25.70 ($C(CH_3)_3$), 43.69 (3′-C), 59.79 (5′-C), 70.79 (4′-C), 77.09 (2′-C), 92.38 (1′-C), 120.45 (5-C), 138.92 (8-C), 149.38 (4-C), 153.04 (2-C), 155.29 (6-C). ESI-HRMS for $[C_{16}H_{27}N_5O_3Si + H]^+$ calcd, 366.1961; found, 366.1956.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (5.17): Following the desilylation method B (which was slightly modified in that the reaction mixture was stirred for 2h at room temperature) **5.15** (210 mg, 0.42 mmol) was converted to **5.17** (140 mg, 87%), which was obtained as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm -0.17 (s, 3H, $SiCH_3$), -0.04 (s, 3H, $SiCH_3$), 0.79 (s, 9H,

$C(CH_3)_3$), 3.55 (dd, *J* = 11.13, 4.98 Hz, 1H, 5′-H), 3.64 (dd, *J* = 11.13, 5.27 Hz, 1H, 5′-H), 4.02 (d, *J* = 8.79 Hz, 1H, 4′-H), 4.08 (d, *J* = 8.79 Hz, 1H, 4′-H), 4.66 (d, *J* = 4.10 Hz, 1H, 2′-H), 4.74 (t, *J* = 5.27 Hz, 1H, 5′-OH), 5.35 (s, 1H, 3′-OH), 5.87 (d, *J* = 4.10 Hz, 1H, 1′-H), 7.27 (br. s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.32 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm -5.30 ($Si(CH_3)_2$), 17.49 ($C(CH_3)_3$), 25.37 ($C(CH_3)_3$), 61.85 (5′-C), 74.59 (4′-C), 79.92 (3′-C) 81.13 (2′-C) 89.86 (1′-C), 118.84 (5-C), 139.70 (8-C), 149.00 (4-C), 152.42 (2-C), 155.90 (6-C). ESI-HRMS for $[C_{16}H_{27}N_5O_4Si + H]^+$ calcd, 382.1911; found, 382.1908.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (5.18): To a solution of compound **5.16** (30 mg, 0.082 mmol) in anh. THF (2 mL) was added CDI (27 mg, 0.164 mmol) and the mixture was stirred at room temperature for 3h. Cold ethylamine (0.3 mL) was added to the reaction mixture and stirring continued at room temperature for 16h. Volatiles were evaporated and the residue suspended in CH_2Cl_2, washed with sat. NH_4Cl, water and brine. The organic layer was dried over anh. Na_2SO_4, filtered, evaporated. The residue was purified by column chromatography (1-3% MeOH in CH_2Cl_2) to afford the title compound (35 mg, 98%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.12 (s, 3H, $SiCH_3$), 0.18 (s, 3H, $SiCH_3$), 0.92 (s, 9H, $C(CH_3)_3$), 1.12 (t, *J* = 7.18 Hz, 3H, NCH_2CH_3) 2.61 - 2.80 (m, 1H, 3′-H), 3.20 (quin, *J* = 6.59 Hz, 2H, NCH_2CH_3), 4.05 (t, *J* = 8.79 Hz, 1H, 4′-H), 4.14 - 4.34 (m, 2H, 5′-H), 4.44 (t, *J* = 8.05 Hz, 1H, 4′-H), 4.62 (t, *J* = 4.83 Hz, 1H, NH), 4.87 (d, *J* = 4.10 Hz, 1H, 2′-H), 5.65 (br.s, 2H, NH_2), 5.87 - 5.96 (d, *J* = 1.17 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.29, -4.59 ($Si(CH_3)_2$), 15.25 (NCH_2CH_3), 18.00 ($C(CH_3)_3$), 25.72 ($C(CH_3)_3$), 35.89 (NCH_2CH_3), 41.75 (3′-C), 61.03 (5′-C), 71.26 (4′-C), 75.99 (2′-C), 92.67 (1′-C), 120.44 (5-C), 138.51 (8-C), 149.24 (4-C), 153.00 (2-C), 155.30 (6-C), 155.97 (CO). ESI-HRMS for $[C_{19}H_{32}N_6O_4Si]$ calcd, 437.2333; found, 437.2338.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (5.19): Following the same protocol as described for **5.18**, 30 mg (0.078 mmol) of **5.17** was converted to **5.19** (17 mg, 52%), obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.01 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.91 (s, 9H,

$C(CH_3)_3$), 1.14 (t, *J* = 7.18 Hz, 3H, NCH_2CH_3), 3.24 (qd, *J* = 7.32, 1.17 Hz, 2H, NCH_2CH_3) 4.06 (d, *J* = 9.37 Hz, 1H, 4′-H), 4.17 (d, *J* = 9.37 Hz, 1H, 4′-H), 4.32 (d, *J* = 11.42 Hz, 1H, 5′-H), 4.41 (d, *J* = 11.42 Hz, 1H, 5′-H), 4.56 (s, 1H, 2′-H), 4.84 (t, *J* = 5.13 Hz, 1H, CONH), 5.75 (s, 1H, 1′-H), 5.83 (br. s, 2H, NH_2), 7.95 (s, 1H, 8-H), 8.32 (s, 1H, 2-H). ^{13}C NMR (75 MHz, CDCl3) δ ppm -5.22 ($SiCH_3$), -4.44 ($SiCH_3$), 15.17 (NCH_2CH_3), 17.81 ($C(CH_3)_3$), 25.61 ($C(CH_3)_3$), 35.99 (NCH_2CH_3), 65.30 (5′-C), 76.41 (4′-C), 80.48 (3′-C), 82.81 (1′-C), 94.41 (1′-C), 120.43 (5-C), 140.41 (8-C), 148.43 (4-C), 152.50 (2-C), 155.70 (6-C), 156.44 (CO). ESI-HRMS for $[C_{19}H_{32}N_6O_5Si + H]^+$ calcd, 453.2282; found, 453.2220.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-(*N*-ethyl)-carboxamido-α-D-apio-L-furanose (5.20): To a suspension of compound **5.16** (30 mg, 0.082 mmol) in a 1:1:1.5 mixture of acetonitrile-CCl_4-water (2 mL) was added $NaIO_4$ (75 mg, 0.33 mmol), followed by $RuCl_3$ (12 mg). The reaction mixture was stirred at room temperature for 7h and the solvents evaporated under vacuum. The residue was suspended in MeOH (5 mL), subjected to centrifugation and the clear supernatant liquid was decanted and evaporated. The residue was dried under high vacuum, and suspended in anh. THF (1.5 mL). To this suspension was added DMAP (23 mg, 0.19 mmol) and CDI (27 mg, 0.17 mmol) and the mixture was stirred at room temperature for 2-3h. Cold ethylamine (0.3 mL) was added, stirring was continued at room temperature for 18h, after which the reaction mixture was evaporated and the residue purified by column chromatography. The products were further purified by preparative thin layer chromatography (10% MeOH in CH_2Cl_2) to afford the title compound **5.20** (R_f: 0.4, 3 mg, 9%), as well as the carbamate **5.18** (R_f: 0.55, 3 mg, 8%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.01 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.87 (s, 9H, $C(CH_3)_3$), 1.18 (t, *J* = 7.32 Hz, 3H, NCH_2CH_3), 3.14 - 3.23 (m, 1H, 3′-H), 3.27 - 3.44 (m, 2H, NCH_2CH_3), 4.51 (dd, *J* = 8.79, 6.74 Hz, 1H, 4′-H), 4.57 (dd, *J* = 8.64, 7.18 Hz, 1H, 4′-H), 5.33 (dd, *J* = 5.86, 3.22 Hz, 1H, 2′-H), 5.64 (br. s, 2H, 6-NH_2), 5.91 (d, *J* = 3.51 Hz, 1H, 1′-H), 6.09 (t, *J* = 4.54 Hz, 1H, 5′-NH), 7.86 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ESI-HRMS for $[C_{18}H_{30}N_6O_3Si + H]^+$ calcd, 407.2227; found, 407.2222.

1′-(Adenin-9-yl)-3′-deoxy-5′-(*N*-ethyl)-carboxamido-α-D-apio-L-furanose (5.21): To a solution of compound **5.20** (3 mg, 0.007 mmol) in MeOH (0.5 mL) in a polypropylene vessel was added NH_4F (6 mg, 0.14 mmol) and the reaction mixture was stirred at 50 °C for 48h. After addition of CH_2Cl_2 (1.5 mL) and the reaction mixture was filtered through celite pad. The filtrate was concentrated and the residue purified by column chromatography (5-7 % MeOH in CH_2Cl_2) to afford the title compound **5.21** (1.5 mg, 70 %) as a white solid after lyophylization. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.02 (t, J = 7.18 Hz, 3H, NCH_2CH_3), 3.04 - 3.15 (m, 2H, NCH_2CH_3), 4.23 (t, J = 8.20 Hz, 1H, 4′-H), 4.35 (t, J = 8.05 Hz, 1H, 4′-H), 4.77 (br. s, 1H, 2′-H), 5.88 (d, J = 3.51 Hz, 1H, 2′-OH), 5.95 (d, J = 2.64 Hz, 1H, 1′-H), 7.26 (s, 2H, 6-NH_2), 7.89 (t, J = 5.42 Hz, 1H, 5′-NH), 8.15 (s, 1H, 2-H), 8.23 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 14.64 (NCH_2CH_3), 33.43 (NCH_2CH_3), 47.11 (3′-C), 69.12 (4′-C), 75.17 (2′-C), 90.38 (1′-C), 119.19 (5-C), 138.83 (8-C), 148.91 (4-C), 152.56 (2-C), 156.01 (6-C), 168.19 (CO). ESI-HRMS $[C_{12}H_{16}N_6O_3 + H]^+$ calcd, 293.1362; found, 293.1360.

1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (5.22): To a solution of compound **5.20** (35 mg, 0.08 mmol) in MeOH (1.5 mL) in a polypropylene vessel was added NH_4F (60 mg, 1.6 mmol) and the reaction mixture was stirred at 50 °C for 48h. After addition of CH_2Cl_2 (5 mL) and the reaction mixture was filtered through celite pad. The filtrate was concentrated and the residue purified by column chromatography (5-7 % MeOH in CH_2Cl_2) to afford the title compound **5.22** (23 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.00 (t, J = 7.20 Hz, 3H, NCH_2CH_3), 2.73 - 2.87 (m, 1H, 3′-H), 2.93 - 3.05 (m, 2H, NCH_2CH_3), 3.85 (t, J = 8.35 Hz, 1H, 4′-H), 4.03 (dd, J = 10.56, 8.25 Hz, 1H, 5′-H), 4.23 (dd, J = 10.85, 6.24 Hz, 1H, 5′-H), 4.37 (t, J = 7.77 Hz, 1H, 4′-H), 4.66 (t, J = 3.55 Hz, 1H, 2′-H), 5.86 (d, J = 4.80 Hz, 1H, 2′-OH), 5.92 (d, J = 2.11 Hz, 1H, 1′-H), 7.10 (t, J = 5.37 Hz, 1H, NH), 7.27 (br.s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.23 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 15.01 (NCH_2CH_3), 35.00 (NCH_2CH_3), 41.19 (3′-C), 60.76 (5′-C), 70.59 (4′-C), 74.24 (2′-C), 91.29 (1′-C), 119.24 (5-C), 139.05 (8-C), 148.73 (4-C), 152.55 (2-C), 155.99 (CO), 156.02 (6-C). ESI-HRMS for $[C_{13}H_{18}N_6O_4 + H]^+$ calcd, 323.1468; found, 323.1472.

1′-(Adenin-9-yl)-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (5.23): Following the protocol used for the synthesis of **5.22**, compound **5.21** (20 mg, 0.044 mmol) was converted to the title compound **5.23** (14 mg, 94%), obtained as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.11 (t, *J* = 7.18 Hz, 3H, NCH_2CH_3), 3.14 (q, *J* = 7.03 Hz, 2H, NCH_2CH_3), 4.13 (d, *J* = 9.37 Hz, 1H, 4′-H), 4.20 (d, *J* = 9.37 Hz, 1H, 4′-H), 4.26 (d, *J* = 11.42 Hz, 1H, 5′-H), 4.33 (d, *J* = 11.13 Hz, 1H, 5′-H), 4.37 (d, *J* = 1.17 Hz, 1H, 2′-H), 6.04 (d, *J* = 1.76 Hz, 1H, 1′-H), 8.21 (s, 1H, 2-H), 8.31 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 15.33 (NCH_2CH_3), 36.70 (NCH_2CH_3), 66.37 (5′-C), 77.43 (4′-C), 81.34 (3′-C), 81.93 (2′-C), 93.89 (1′-C), 120.30 (5-C), 141.53 (8-C), 150.00 (4-C) 153.69 (2-C), 157.35 (6-C), 158.83 (CO). ESI-HRMS for $[C_{13}H_{18}N_6O_5 + H]^+$ calcd, 339.1417; found, 339.1409.

1′-[N^6-(3-Chlorobenzyl)-adenin-9-yl]-β-D-apio-D-furanose (5.24): Following the protocol used for the synthesis of compound **5.5**, β-D-apio-D-furanoadenosine **4.3b** (100 mg, 0.38 mmol) was converted to the title compound, which was first purified by silica-gel coumn chromatography and then by RP-HPLC (H_2O/AcCN, 90/10→70/30 in 18 min, R_t = 12.5 min) to obtain **5.24** as white solid (16 mg, 11%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.46 (q, *J* = 11.02 Hz, 2H, 5′-H), 3.77 (d, *J* = 9.25 Hz, 1H, 4′-H), 4.32 (d, *J* = 9.05 Hz, 1H, 4′-H), 4.72 (br. s, 2H, $PhCH_2$), 4.82 (d, *J* = 7.48 Hz, 1H, 2′-H), 4.87 (br.s, 1H, 3′-OH), 5.43 (br.s, 1H, 2′-OH), 5.90 (d, *J* = 7.67 Hz, 1H, 1′-H), 7.23 - 7.43 (m, 4H, $PhCH_2$), 8.23 (s, 1H, 2-H), 8.38 (s, 1H, 8-H), 8.44 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 42.41 ($PhCH_2$), 62.38 (5′-C), 73.42 (2′-C), 74.59 (4′-C), 78.28 (3′-C), 87.81 (1′-C), 119.70 (5-C) 125.80, 126.58, 126.88, 130.14, 132.87 ($PhCH_2$), 140.21 (8-C), 142.78 ($PhCH_2$), 149.18 (4-C), 152.56 (2-C), 154.33 (6-C). ESI-HRMS for $[C_{17}H_{18}ClN_5O_4 + H]^+$ calcd, 392.1120; found, 392.1127.

1′-[N^6-(3-Iodobenzyl)-adenin-9-yl]-β-D-apio-D-furanose (5.25): Following the protocol used for the synthesis of compound **5.5**, β-D-apio-D-furanoadenosine **4.3b** (100 mg, 0.38 mmol) was converted to the title compound **5.25**, which was first purified by silica-gel coumn chromatography and then by trituration with methanol to obtain as white solid (15 mg, 8%).^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.37 - 3.57 (m, 2H, 5′-CH_2), 3.76 (d, *J* = 9.21 Hz, 1H, 4′-H), 4.32 (d, *J* = 9.21 Hz, 1H, 4′-H), 4.67

(br. s, 2H, PhCH_2), 4.76 - 4.82 (m, 1H, 2′-H), 4.84 (s, 1H, 3′-OH), 4.90 (t, *J* = 5.49 Hz, 1H, 5′-OH), 5.40 (d, *J* = 6.86 Hz, 1H, 2′-H), 5.89 (d, *J* = 7.45 Hz, 1H, 1′-H), 7.11(t, *J* = 7.64 Hz, 1H, *Ph* 5-H), 7.36 (d, *J* = 7.64 Hz, 1H, *Ph*) 7.58 (d, *J* = 7.84 Hz, 1H, *Ph*) 7.72 (s, 1H, *Ph* 2-H), 8.22 (s, 1H, 2-H), 8.38 (s, 1H, 8-H), 8.41 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 42.24 (Ph*C*H_2), 62.37 (5′-C), 73.39 (2′-C), 74.57 (4′-C), 78.26 (3′-C), 87.80 (1′-C), 94.71 (*Ph*), 119.72 (5-C), 126.59, 130.47, 135.32, 135.68 (*Ph*), 140.17 (8-C) 142.88 (*Ph*), 149.13 (4-C), 152.54 (2-C), 154.31 (6-C). ESI-HRMS for $[C_{17}H_{18}IN_5O_4 + H]^+$ calcd, 484.0476; found, 484.0490.

1′-[*N*^{6}-(2-Methoxy-5-chlorobenzyl)-adenin-9-yl]-β-D-apio-D-furanose (5.26): To a solution of apioadenosine **4.53b** (60 mg, 0.23 mmol) in anh. DMF (2 mL) was added 2-methoxy-5-chlorobenzyl bromide (58 mg, 0.25 mmol) and the mixture was stirred at room temperature for 24h. Solvent was evaporated in vacuo. The residue was trated with ammonium hydroxide (25%, 3.0 mL) and stirred at 55 °C for 24h. The volatiles were evaporated under reduced pressure and the residue purified by silica-gel column chromatography (3-6% MeOH in CH_2Cl_2) and the products were further purified by preperative thin layer chromatography to afford desired product **5.26** (20 mg, 21%) as white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.59 - 3.74 (q, *J* = 11.37 Hz, 2H, 5′-H), 3.87 (s, 3H, OCH_3), 3.98 (d, *J* = 9.63 Hz, 1H, 4′-H), 4.49 (d, *J* = 9.63 Hz, 1H, 4′-H), 4.77 (br.s, 2H, PhCH_2), 4.85 (d, *J* = 7.13 Hz, 1H, 2′-H), 6.01 (d, *J* = 7.13 Hz, 1H, 1′-H), 6.93 (d, *J* = 8.67 Hz, 1H, Ph 5-H), 7.20 (dd, *J* = 8.67, 2.70 Hz, 1H, Ph 4-H), 7.25 (d, *J* = 2.50 Hz, 1H, Ph 2-H), 8.25 (s, 2H, 2-H & 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 40.25 (Ph*C*H_2), 56.25 (O*C*H_3), 64.42 (5′-C), 75.99 (2′-C), 76.42 (4′-C), 79.85 (3′-C), 90.47 (1′-C), 112.79 (Ph) 120.99 (5-C), 126.27, 129.03, 129.05, 129.96 (Ph), 141.13 (8-C), 150.25 (4-C), 153.97 (2-C), 156.01 (6-C), 157.52 (Ph). ESI-HRMS for $[C_{18}H_{20}ClN_5O_5 + H]^+$ calcd, 422.1226; found, 422.1235.

1′-(Adenin-9-yl)-2′,5′-*O*-di(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (5.27): Following the reaction protocol used for the synthesis of compound **5.14**, β-D-apio-D-furanoadenosine **4.3b** (150 mg, 0.56 mmol) was converted to the title compound **5.27** (207 mg, 74%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.47, -0.07, 0.10, 0.12 (s's, 4 x 3H, SiCH_3), 0.81, 0.97 (s's, 9H, C(CH_3)$_3$), 3.01 (s, 1H, 3′-OH), 3.50 (d,

J = 10.26 Hz, 1H, 5′-H), 3.59 (d, *J* = 10.26 Hz, 1H, 5′-H), 4.02 (d, *J* = 9.43 Hz, 1H, 4′-H), 4.50 (dd, *J* = 9.43, 1.03 Hz, 1H, 4′-H), 5.41 (d, *J* = 6.56 Hz, 1H, 2′-H), 5.65 (br.s, 2H, NH_2), 5.89 (d, *J* = 6.77 Hz, 1H, 1′-H), 7.86 (s, 1H, 2-H), 8.34 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.68, -5.47, -5.22, -5.02 (Si*C*H_3), 17.76, 18.13 (*C*$(CH_3)_3$) 25.54, 25.73 (C(*C*$H_3)_3$), 62.13 (5′-C), 73.95 (2′-C), 74.11 (4′-C), 78.79 (3′-C), 89.89 (1′-C), 120.43 (5-C), 140.03 (8-C), 150.11 (4-C), 153.23 (2-C), 155.46 (6-C). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2781.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (5.28): Following the desilylation method B (which was slightly modified in that the reaction mixture was stirred for 3h at room temperature) **5.27** (200 mg, 0.4 mmol) was converted to **5.28** (90 mg, 35%), which was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.33, -0.03 (s's, 2 x 3H, SiC*H*$_3$), 0.84 (s, 9H, C(C*H*$_3)_3$), 3.28 (s, 1H, 3′-OH), 3.78 (s, 2H, 5′-H), 4.04 (d, *J* = 9.71 Hz, 1H, 4′-H), 4.45 (d, *J* = 9.71 Hz, 1H, 4′-H), 5.25 (d, *J* = 5.26 Hz, 1H, 2′-H), 5.71 (br.s, 2H, NH_2), 5.78 (d, *J* = 5.26 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.36 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.91, 0.15 (Si*C*H_3), 17.96 (*C*$(CH_3)_3$), 25.69 (C(*C*$H_3)_3$), 65.64 (5′-C), 75.56 (2′-C), 75.74 (4′-C), 78.33 (3′-C), 91.67 (1′-C), 120.72 (5-C), 140.57 (8-C), 149.76 (4-C), 153.35 (2-C), 155.78 (6-C). ESI-HRMS for $[C_{16}H_{27}N_5O_4Si + H]^+$ calcd, 382.1911; found, 382.1910.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*N*-methylcarbamoyl)-β-D-apio-D-furanose (5.29): Following the same protocol as described for **5.18**, 85 mg (0.22 mmol) of **5.28** was converted to **5.29** (70 mg, 72%), obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm -0.42, -0.05 (s's, 3H, SiC*H*$_3$), 0.83 (s, 9H, C(C*H*$_3)_3$), 2.85 (d, *J* = 4.90 Hz, 3H, NC*H*$_3$), 3.26 (br.s, 1H, 3′-OH), 4.09 (d, *J* = 9.81 Hz, 1H, 4′-H), 4.18 (d, *J* = 11.44 Hz, 1H, 5′-H), 4.28 (d, *J* = 11.44 Hz, 1H, 5′-H), 4.46 (d, *J* = 9.81 Hz, 1H, 4′-H), 4.90 (br.s, 1H, NH), 5.32 (d, *J* = 6.13 Hz, 1H, 2′-H), 5.81 (d, *J* = 6.13 Hz, 1H, 1′-H), 5.89 (br.m, 2H, NH_2), 7.86 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.14, -5.09 ($SiCH_3$), 17.76 (*C*$(CH_3)_3$), 25.52 (C(*C*$H_3)_3$), 27.71 (NCH_3), 65.43 (5′-C), 74.99 (2′-C), 75.13 (4′-C), 90.60 (1′-C),

120.60 (5-C), 140.49 (8-C), 149.86 (4-C), 153.17 (2-C), 155.65 (6-C), 156.60 (C=O). ESI-HRMS for $[C_{18}H_{30}N_6O_5Si + H]^+$ calcd, 439.2125; found, 439.2128.

1′-(Adenin-9-yl)-5′-*O*-(*N*-methylcarbamoyl)-β-D-apio-D-furanose (5.30): Following the protocol used for the synthesis of **5.22**, compound **5.29** (60 mg, 0.13 mmol) was converted to the title compound **5.30** (40 mg, 90%), obtained as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 2.73 (s, 3H, NCH_3), 3.99 (d, *J* = 9.63 Hz, 1H, 4′-H), 4.15 - 4.31 (q, *J* = 11.18 Hz, 2H, 5′-H), 4.46 (d, *J* = 9.83 Hz, 1H, 4′-H), 4.91 (d, *J* = 7.13 Hz, 1H, 2′-H), 5.99 (d, *J* = 7.13 Hz, 1H, 1′-H), 8.21 (s, 1H, 2-H), 8.28 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 27.53 (NCH_3), 66.69 (5′-C), 76.15 (2′-C), 76.43 (4′-C), 78.55 (3′-C), 90.40 (1′-C), 120.72 (5-C), 141.79 (8-C), 150.86 (4-C), 153.92 (2-C), 157.33 (6-C), 159.21 (C=O). RSI-HRMS for $[C_{12}H_{16}N_6O_5 + H]^+$ calcd, 325.1255; found, 325.1260.

5.4.2. Pharmacological assay procedures

Receptor binding and functional assays

[^{3}H]R-*N*^{6}-Phenylisopropyladenosine ([^{3}H]R-PIA, 63 Ci/mmol), [^{125}I]*N*^{6}-(4-Amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide ([^{125}I]I-AB-MECA, 2200 Ci/mmol), and [^{3}H](2-[p-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamido-adenosine) ([^{3}H]CGS21680, 40.5 Ci/mmol) were purchased from Perkin–Elmer Life and Analytical Science (Boston, MA). Test compounds were prepared as 5 mM stock solutions in DMSO and stored frozen.

Cell Culture and Membrane Preparation - CHO cells stably expressing the recombinant hA_1, hA_3, and rA_3Rs, and HEK-293 cells stably expressing the $hA_{2A}AR$ were cultured in Dulbecco's modified Eagle medium (DMEM) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 μmol/mL glutamine. In addition, 800 μg/mL geneticin was added to the A_{2A} media, while 500 μg/mL hygromycin was added to the A_1 and A_3 media. After harvesting, cells were homogenized and suspended in PBS. Cells were then

centrifuged at 240 *g* for 5 min, and the pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgCl_2$. The suspension was homogenized and was then ultra-centrifuged at 14,330 *g* for 30 min at 4 °C. The resultant pellets were resuspended in Tris buffer, incubated with adenosine deaminase (3 units/mL) for 30 min at 37 ^{o}C. The suspension was homogenized with an electric homogenizer for 10 sec, pipetted into 1 mL vials and then stored at -80 °C until the binding experiments. The protein concentration was measured using the BCA Protein Assay Kit from Pierce Biotechnology, Inc. (Rockford, IL).

Binding assays: Into each tube in the binding assay was added 50 μL of increasing concentrations of the test ligand in Tris-HCl buffer (50 mM, pH 7.5) containing 10 mM $MgCl_2$, 50 μL of the appropriate agonist radioligand, and finally 100 μL of membrane suspension. For the A_1AR (22 μg of protein/tube) the radioligand used was [^{3}H]R-PIA (final concentration of 3.5 nM). For the A_{2A}AR (20 μg/tube) the radioligand used was [^{3}H]CGS21680 (10 nM). For the A_3AR (21 μg/tube) the radioligand used was [^{125}I]I-AB-MECA (0.34 nM). Nonspecific binding was determined using a final concentration of 10 μM adenosine-5′-*N*-ethylcarboxamide (NECA) diluted with the buffer. The mixtures were incubated at 25 °C for 60 min in a shaking water bath. Binding reactions were terminated by filtration through Brandel GF/B filters under a reduced pressure using a M-24 cell harvester (Brandel, Gaithersburg, MD). Filters were washed three times with 3 mL of 50 mM ice-cold Tris-HCl buffer (pH 7.5). Filters for A_1 and A_{2A}AR binding were placed in scintillation vials containing 5 mL of Hydrofluor scintillation buffer and counted using a Perkin Elmer Liquid Scintillation Analyzer (Tri-Carb 2810TR). Filters for A_3AR binding were counted using a Packard Cobra II γ-counter. The K_i values were determined using GraphPad Prism for all assays.

cAMP accumulation assay: Intracellular cAMP levels were measured with a competitive protein binding method. CHO cells that expressed the recombinant hA_3AR were harvested by trypsinization. After centrifugation and resuspended in medium, cells were planted in 96-well plates in 0.1 mL medium. After 24h, the medium was removed and cells were washed three times with 0.2 mL DMEM,

containing 50 mM HEPES, pH 7.4. Cells were then treated with the agonist NECA or test compound in the presence of rolipram (10 μM) and adenosine deaminase (3 units/mL). After 30 min forskolin (10 μM) was added to the medium, and incubation was continued for an additional 15 min. The reaction was terminated by removing the supernatant, and cells were lysed upon the addition of 100 μL of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at -20°C. For determination of cAMP production, 50 μL of the HCl solution was used in the Amersham cAMP Enzyme Immunoassay following the instructions provided with the kit. The results were interpreted using a SpectroMax M5 Microplate reader at 450 nm.

CHAPTER – 6

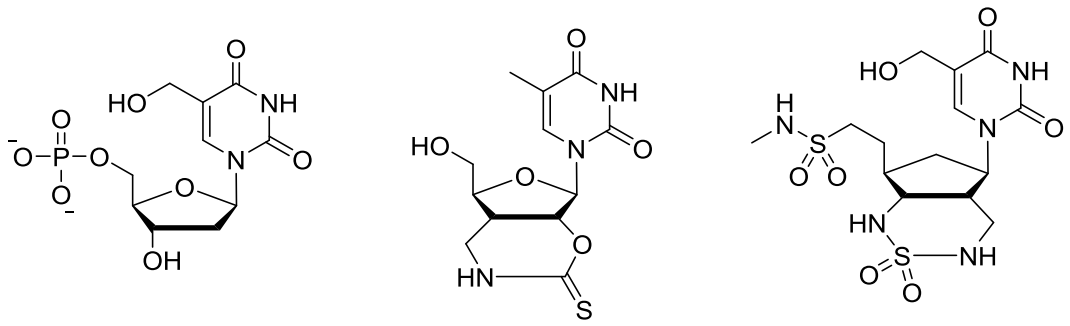
5,5'-MODIFIED 2'-DEOXYURIDINES AS TMPK*mt* INHIBITORS

Part of this Chapter was published as

Synthesis and evaluation of 5′-modified thymidines and 5-hydroxymethyl-2′-deoxyuridines as *M. tuberculosis* thymidylate kinase inhibitors.
Kiran S. Toti, Frederick Verbeke, Martijn D. P. Risseeuw, Vladimir Frecer, Hélène Munier-Lehmann, Serge Van Calenbergh* *Bioorganic & Medicinal Chemistry*, **2013**, *21*, 257-268.

6.1. Objectives

Frecer *et al.* recently reported on the combinatorial design and structure-based *in silico* screening of a virtual focused library of bicyclic thymidine analogs.[154] In their study they used the three-dimensional structure of TMPK*mt* complexed with 5-hydroxymethyl-dUMP (**6.1**) to develop a QSAR model, to parameterize a target-specific scoring function for TMPK*mt* and to select virtual hits which display the highest predicted binding to the target. Compound **6.3** emerged as one of the best analogues. The binding of **6.3** to TMPK*mt* is predicted to be similar to that of **6.2**. Beside the fact that it is a carba-nucleoside contains a sulfondiamide group replacing the thiourethane moiety of the condensed ring of **6.2**, **6.3** differs from **6.2** by the presence of a 5-CH_2OH group, known to possibly make an extra hydrogen bond with a water molecule (W12) or with the side chain of Ser99, and a 5′-(N-methylsulfamoyl)methyl moiety. The latter fragment, which replaces the phosphate moiety present in dTMP, was anticipated to favorably interact Arg95, Arg153 and especially with the magnesium ion. Alternative phosphate isosteres suggested in the Frecer publication are a 5-tetrazolylmethyl and methylsulfonylmethyl group. In this pilot study we decided to explore the importance of the proposed 5-CH_2OH and 5′-modifications for TMPK*mt* inhibition.



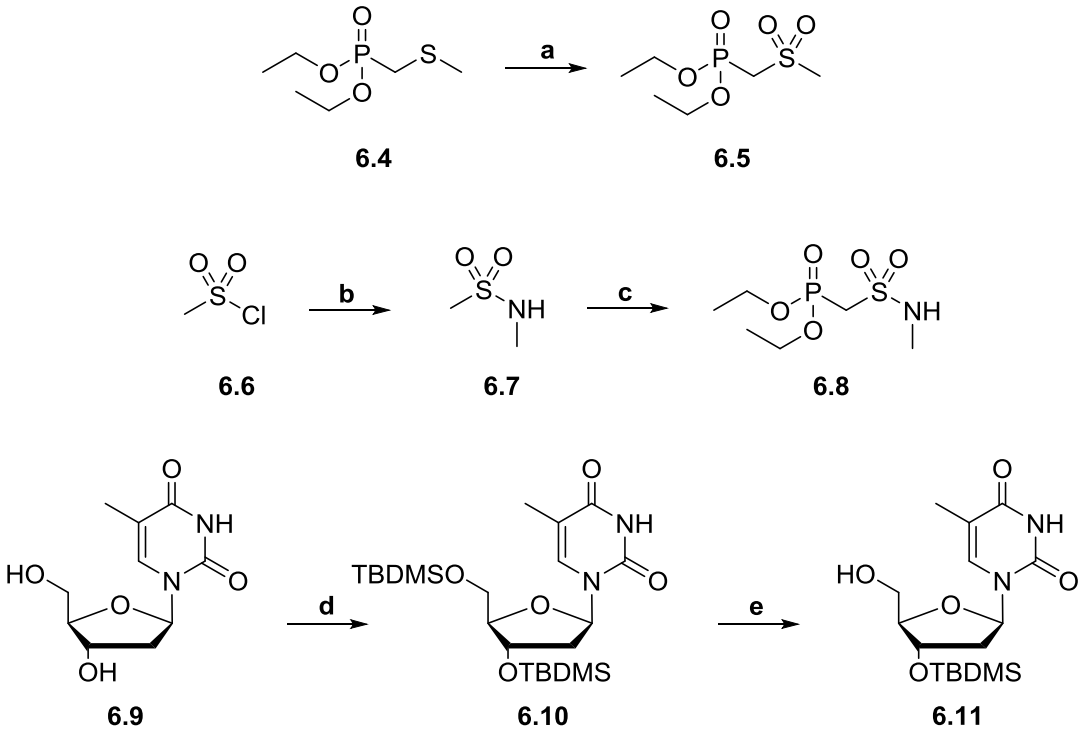
6.1: K_i^{exp}: 110 μM **6.2:** K_i^{exp}: 3.5 μM **6.3:** K_i^{pre}: 0.29 μM

Figure 5.1. TMPK*mt* inhibitors designed previously in our laboratory (**6.1** and **6.2**) or predicted by QSAR model (**6.3**). K_i^{exp} and K_i^{pre} indicate experimental and predicted K_i values, respectively.

6.2. Results and Discussion

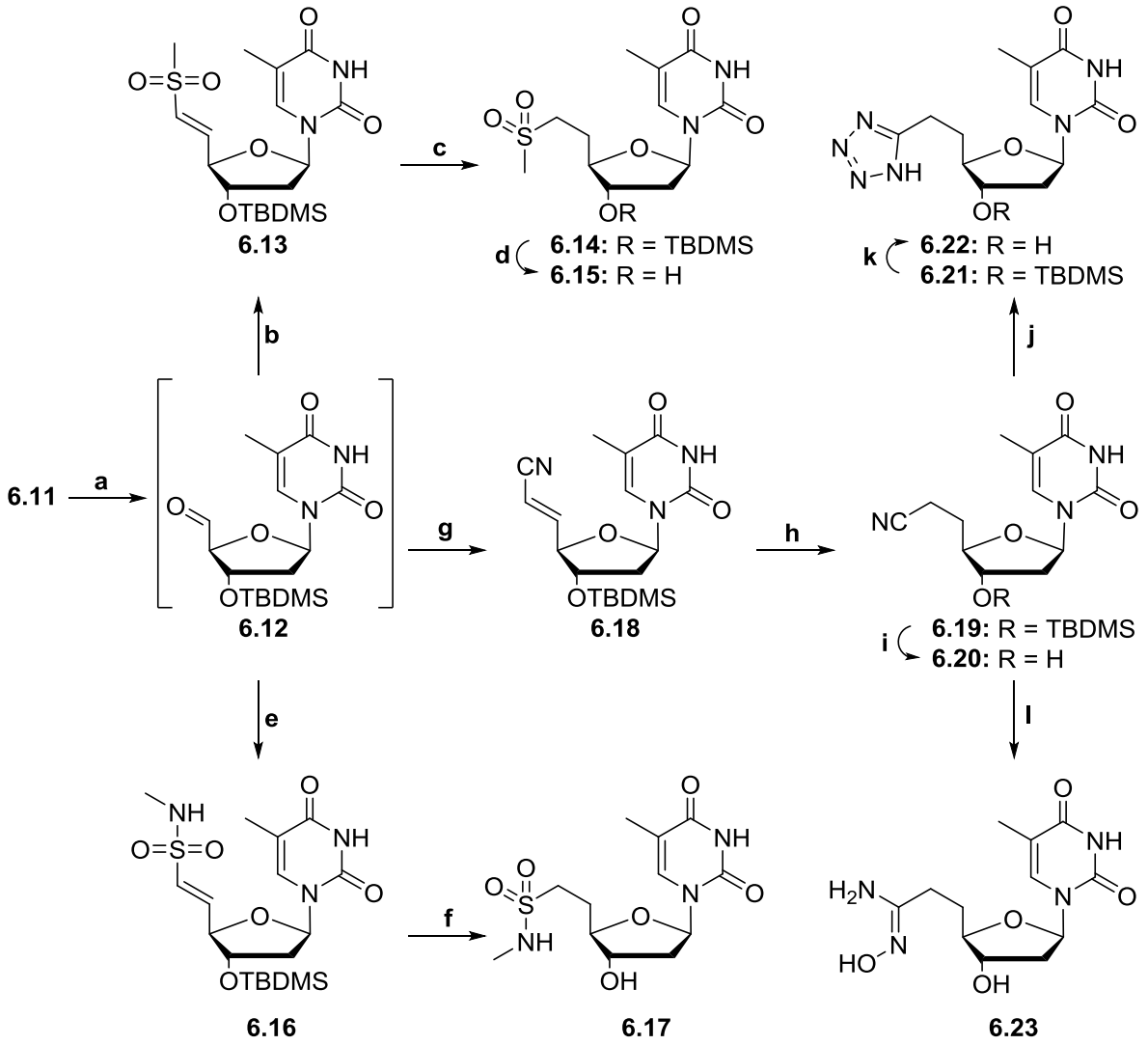
6.2.1. Chemistry

We envisioned synthesizing the target molecules using a Wittig-Horner reaction to introduce the desired 5′-modifications as a key step. For this purpose two non-commercial Horner reagents were self-made (Scheme 6.1). Diethyl ((methylsulfonyl)methyl)phosphonate **6.5** [190] was prepared according to known procedures, while Horner reagent **6.8**[191] was obtained by reacting half an equivalent of chlorodiethylphosphate with bis-lithiated **6.7**. [192] 3′-Silylated thymidine **6.11** was produced by a selective cleavage of the primary TBDMS[183] group of **6.10**.[193]



6.4 **6.5** **6.6** **6.7** **6.8** **6.9** **6.10** **6.11**

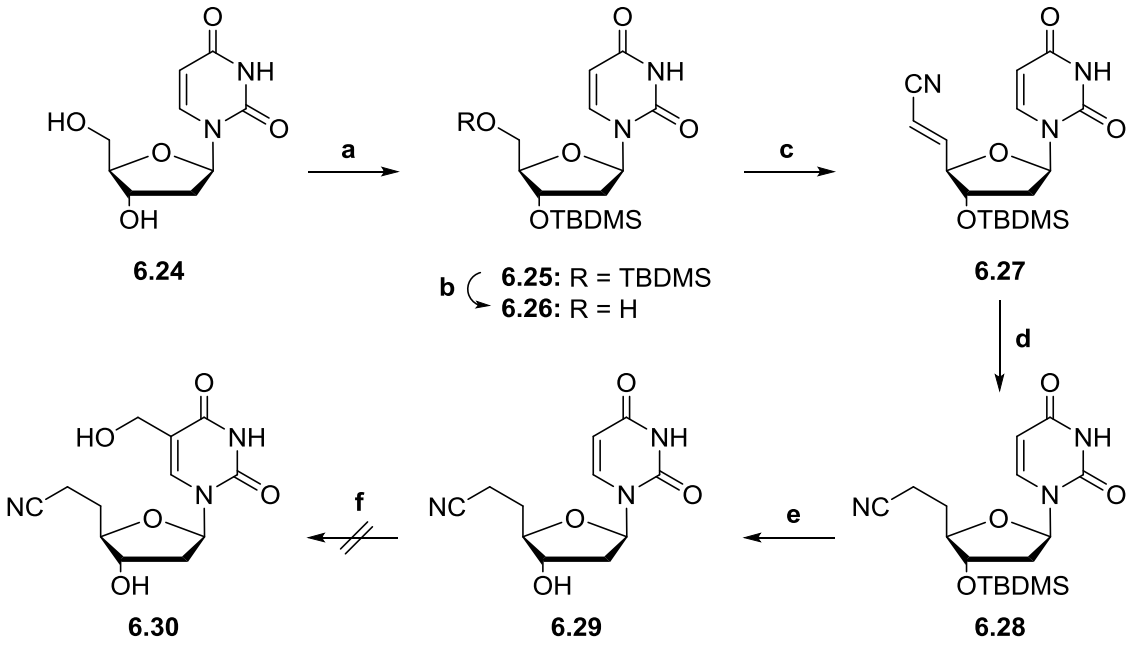
Scheme 6.1. Synthesis of Horner reagents **6.5** and **6.8**, and intermediate **6.11.** *Reagents and conditions*:(a) CH_2Cl_2, m-CPBA, rt, 48h, 99%; (b) aq.methylamine, H_2O, 0 °C → rt, 18h, 92%; (c) THF, BuLi, -78 °C, 1h, diethylchlorophosphate (0.5 eq), -78 °C → 0 °C, 1h, 65 %; (d) DMF, TBDMSCl, imidazole, rt, 18h, quant. yield; (e) THF, pyridine, HF-pyridine, 0 °C, 1h, 0 °C → rt, 1h, 36 %.



Scheme 6.2. Synthesis of the 5′-modified thymidines**.** *Reagents and conditions*: (a) CH_2Cl_2, Dess-Martin periodinane, rt, 4h; (b) THF, **6.5**, BuLi, -78 °C, 15 min, -78 °C, 1h, rt, 18h, 20%; (c) H_2, Pd-C, MeOH, 4h, rt; (d) THF, TBAF, 40 °C, 15h, 20% over two steps; (e) THF, **6.8**, BuLi, -78 °C, 1h, -78 °C, 1h, rt, 18h, 48%; (f) (i) MeOH-THF (5:1), $NiCl_2$, $NaBH_4$, 0 °C, 1h; (ii) THF, TBAF, 40 °C, 1h, 12% over two steps; (g)THF, cyanomethyltriphenylphosphonium chloride, BuLi, 0 °C, 30 min, 0 °C → rt, 18h; (h) pyridine-MeOH (3:1), $NaBH_4$, 120 °C, 4h, 53%; (i) THF, TBAF, rt, 4h, 76%; (j) toluene, $TMSN_3$, Bu_2SnO, 110 °C, 4h, 32%; (k) THF, TBAF, rt, 4h, 34%. (l) 50% aq.NH_2OH, ethanol, rt, 48h, 83%.

To synthesize the 5′-modified thymidines **6.15**, **6.17**, **6.20**, **6.22** and **6.23** (Scheme 6.2), the 5′-OH group of **6.11** was oxidized using Dess-Martin periodinane and the resulting aldehyde **6.12** was reacted with the proper Wittig-Horner reagents to give **6.13**, **6.16** and **6.18** in moderate yields. Methylsulfone **6.15** was obtained by Pd catalyzed

hydrogenation of **6.13**, followed by the removal of TBDMS group. The conjugated double bond in **6.16** was reduced using $NaBH_4/NiCl_2$ [194] and the product was desilylated using TBAF affording **6.17**. Conjugate reduction of nitrile **6.18** was achieved with $NaBH_4$ in a pyridine-MeOH mixture at elevated temperature.[195] Deprotection of **6.19** afforded the 6′-nitrile analogue **6.20** in 76% yield. A cycloaddition between nitrile **6.19** and $TMSN_3$ in the presence of dibutyltinoxide gave **6.21** in moderate yield.[196] Even though the conversion of **6.21** to **6.22** was complete (by LCMS), the isolated yield is lowered by the elaborate purification necessary to isolate the product.[197] Compound **6.20** on treatment with hydroxylamine gave *N*-hydroxyamidine **6.23** in good yield.

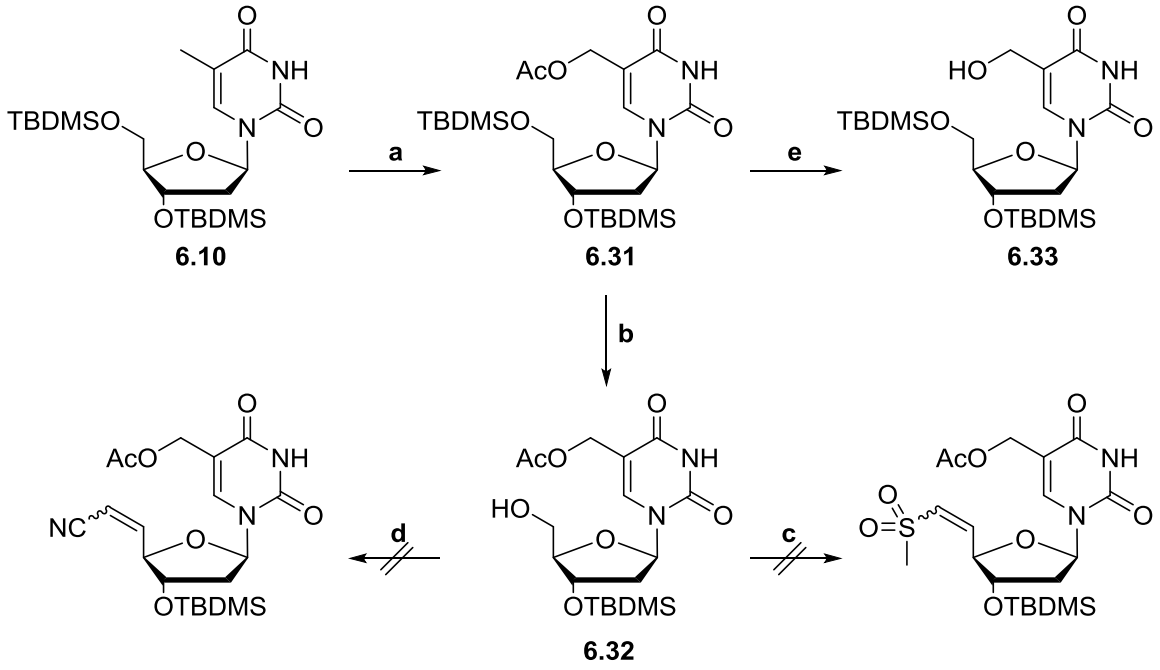


Scheme 6.3. Attempted hydroxymethylation route to **6.30**. *Reagents and conditions*: (a) TBDMSCl, DMF, imidazole, rt, 18h, quant. Yield; (b) THF, pyridine, HF-Pyridine, 0 °C, 1h, 0 °C → rt, 1h, 53 %; (c) (i) CH_2Cl_2, Dess-Martin periodinane, rt, 4h; (ii) THF, cyanomethyltriphenylphosphonium chloride, BuLi, 0 °C, 30 min, 0 °C → rt, 18h, 51% over two steps; (d) H_2, Pd-C, MeOH, 4h, rt; (e) THF, TBAF, rt, 4h, 73%; (f) paraformaldehyde, H_2O, TEA, 100 °C, 3 days or paraformaldehyde, H_2O, TEA, continuous irradiation of microwave from rt → 150 °C in 3 min, 10 min at 150 °C.

Hydroxymethylation at position 5 of dU (Scheme 6.3; **6.24**) of pyrimidine is reported. However, selective protection of this hydroxyl group appears difficult.[198] An attempt

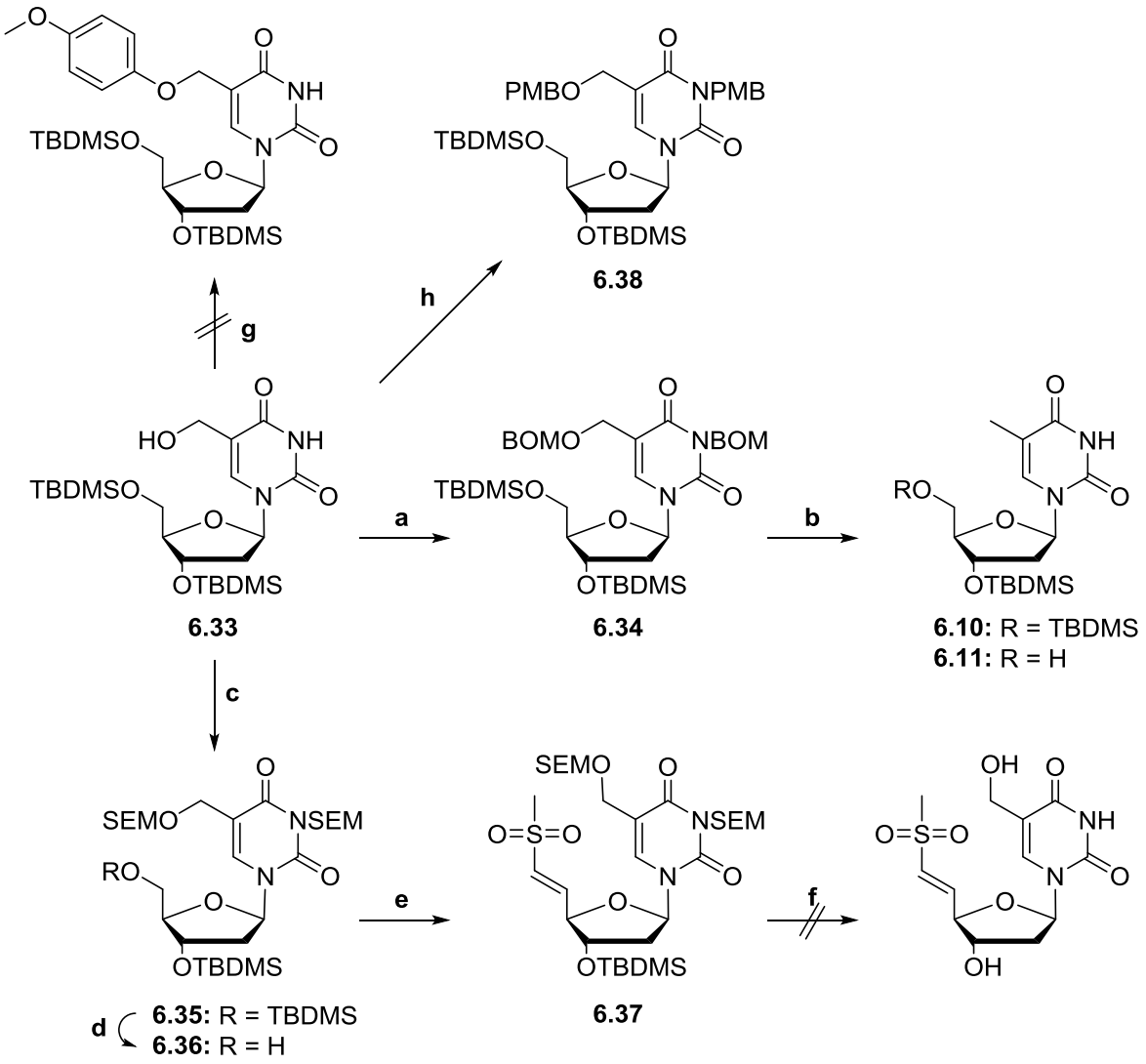
to hydroxymethylate the 5-position of **6.25** failed, possibly due to the low solubility of the substrate in formalin-TEA system and the lability of the TBDMS groups under the harsh reaction conditions. Hence, we decided to introduce the 5-CH_2OH group after modifying the 5′-position in 2′-deoxyuridine. As a model substrate for this strategy, compound **6.29** was synthesized smoothly using similar transformations as before. Unfortunately, reaction of **6.29** with formaldehyde at elevated temperature was very slow with only traces of the desired product formed after several days, while under microwave conditions, the starting material degraded.

Because of this setback, we decided to start from compound **6.31**, obtained via the benzylic oxidation as reported by Grover *et al.* [193] (Scheme 6.4). Selective primary desilylation of **6.31** gave **6.32**. Attempted successive Dess-Martin and Wittig-Horner reactions involving two different Horner reagents failed to give the desired products.



Scheme 6.4. Attempted 5′-derivatization of acetyl protected 2′-deoxy-5-hydroxymethyluridine. *Reagents and conditions*: (a) (i) benzene, NBS, AIBN, 90 °C, 1h; (ii) DMF, CsOAc, rt, 1h, 26% over two steps; (b) THF, pyridine, HF-pyridine, 0 °C, 1h, 0 °C → rt, 1h, 33 %; (c) (i) CH_2Cl_2, Dess-Martin periodinane, rt, 4h; (ii) THF, **6.5**, BuLi, -78 °C, 15 min, -78 °C, 1h, rt; (d) (i) CH_2Cl_2, Dess-Martin periodinane, rt, 4h; (ii) THF, cyanomethyltriphenylphosphonium chloride, BuLi, 0 °C, 30 min, 0 °C → rt. (e) K_2CO_3, MeOH, rt, 6h, 72%.

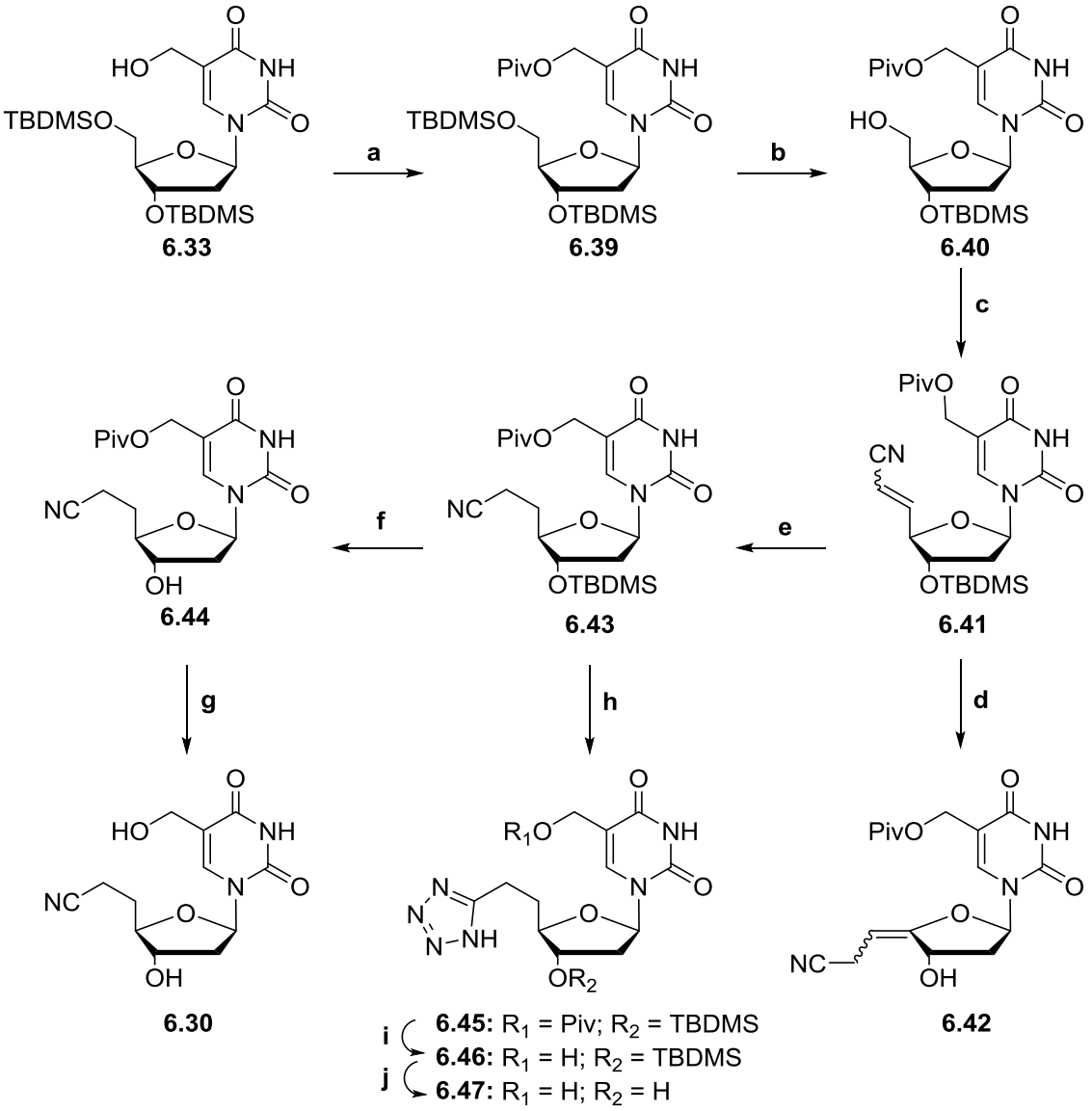
At this point we chose to look for a suitable protecting group for the 5-hydroxymethyl moiety that is compatible with the reaction conditions used to modify the 5′-position and can also be removed under mild conditions. Towards this end 5-hydroxymethyl derivative **6.33** was synthesized according to a literature procedure.[199]



Scheme 6.5. Screening viable protecting group for 5-hydroxymethyl-2′-deoxyuridine. *Reagents and conditions*: (a) DMF, DIPEA, BOM-Cl, 0 °C → rt, 16h, 37 %; (b) MeOH, H_2, Pd-C; (c) CH_2Cl_2, DIPEA, SEM-Cl, 40 °C, 6h, 50%; (d) THF, pyridine, HF-pyridine, 0 °C, 1h, 0 °C → rt, 1h, 38%; (e) (i) CH_2Cl_2, Dess-Martin periodinane, rt, 4h; (ii) THF, **6.5**, BuLi, -78 °C, 15 min, -78 °C, 1h, rt, 16h, 29% over two steps; (f) THF, TBAF, Δ; (g) THF, DEAD, triphenylphosphine, PMP, 0 °C → rt; (h) DMF, PMB-Cl, NaH, 0 °C → rt, 4h, 13%.

BOM derivatization of **6.33** to **6.34** proceeded in 37% yield (Scheme 6.5). Since the desired product also features a benzylic hydroxyl group, we ran a model hydrogenation reaction on **6.34** with palladium as catalyst. Unsurprisingly, the reaction mainly afforded thymidine **6.10**, besides minor amounts (24 %) of **6.11**. Next, the use of a 2-(trimethylsilyl)ethoxymethyl (SEM) protecting group, known to be cleavable under mild conditions, was investigated. Compound **6.33** was converted to **6.35** in moderate yield. Selective removal of the primary TBDMS gave **6.36**, which on DMP oxidation followed by Horner reaction gave **6.37**. Unfortunately, attempts to remove both SEM groups with TBAF in anhydrous THF at elevated temperature failed. Attempts to protect **6.33** as 4-methoxyphenol (PMP) ether under the Mitsunobu conditions led to degradation of the starting material. Conditions used to protect **6.33** with two 4-methoxybenzyl (PMB) groups afforded **6.38** only in low yields (13%), mainly because of simultaneous formation of compounds with three PMB & one TBDMS group and four PMB groups, leaving these protection methods unattractive.

These problems finally led us to protect the 5-CH_2OH group of **6.33** as pivaloate ester in good yield (Scheme 6.6). A series of routine transformations was used to convert **6.39** into the acrylonitrile **6.41** in acceptable yields. Several conditions were evaluated to reduce the double bond in **6.41**, including H_2-Pd-C, $NaBH_4$ in pyridine and $NaBH_4$-$NiCl_2$ in MeOH-THF. Unfortunately, all these methods led to deoxygenation of the 5-CH_2OH group and formation of a thymine base as a predominant side product. Attempts to remove the pivaloyl prior from **6.41** with tetrabutylammmonium hydroxide gave undesired product **6.42**. Hydrogenation using platinum on carbon afforded **6.43** in 70 % yield. Subsequent removal of the TBDMS group with TBAF and the pivaloyl group with sodium methoxide gave **6.30** in excellent yield. Azidotrimethylsilane mediated cycloaddition of nitrile **6.43** gave **6.45** in 74% yield. Removal of the remaining protecting groups with NaOMe in MeOH and NH_4F in MeOH allowed obtaining **6.47** in excellent yield. For the desilylation NH_4F proved to be superior to TBAF with regard to purification of the final product.



Scheme 6.6. Synthesis of the 5′-modified 2′-deoxy-5-hydroxymethyluridine analogs **30** and **47**. *Reagents and conditions*: (a) pyridine, DMAP, Piv-Cl, rt, 18h,74%; (b) THF, pyridine, HF-pyridine, 0 °C, 1h, 0 °C → rt, 1h, 59%; (c) (i) CH_2Cl_2, Dess-Martin periodinane, rt, 4h; (ii) anh. THF, cyanomethyltriphenyl phosphonium chloride, -78 °C, BuLi, 30 min, -78 °C → rt, 18h, 82% over two steps; (d) THF, Bu_4NOH, rt, 5h, 40%; (e) MeOH, H_2, Pt-C, rt, 4h, 70%; (f) THF, TBAF, rt, 4h, 90%; (g) 0.5M NaOMe in MeOH, rt, 3h, 95%; (h) toluene, $TMSN_3$, Bu_2SnO, 110 °C, 4h, 74%; (i) 0.5M NaOMe in MeOH, rt, 3h, 92%; (j) NH_4F, MeOH, 50 °C, 2 days, 87%.

6.2.2. Pharmacological and modelling results

The capacity of compounds **6.15**, **6.17**, **6.20**, **6.22**, **6.23**, **6.30** and **6.47** to inhibit TMPK*mt* was assessed via a spectrophotometric binding assay (K_i).[150] Compound **6.20** (K_i: 48 µM) and **6.22** (K_i: 70 µM) showed the highest activity, while compounds **6.15** (340 µM), **6.17** (240 µM) and **6.23** (140 µM) were comparatively less active. Surprisingly, compounds **6.30** and **6.47**, in which the favorable 5′-modifications were combined with a 5-CH_2OH modification of the nucleobase, failed to inhibit the enzyme at the highest concentration tested (2.6 and 2.4 mM, respectively). Clearly, introduction of the 5-hydroxymethyl group jeopardized the binding of 5′-modified analogues to TMPK*mt*.

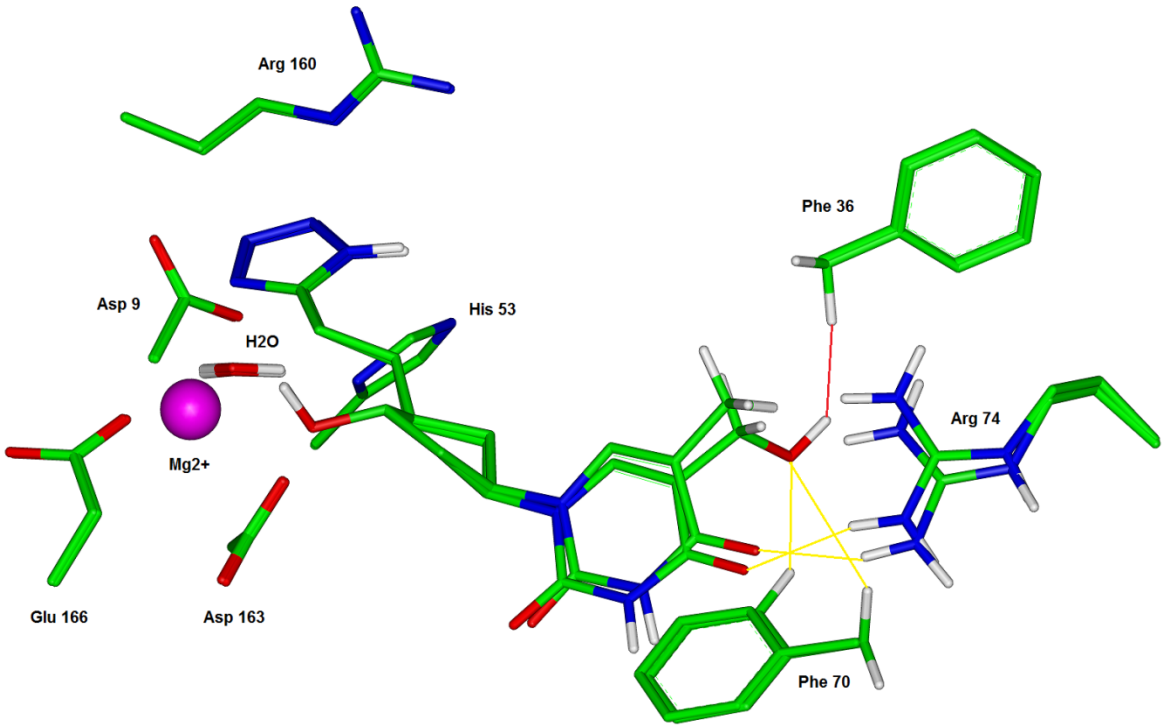


Figure 6.2. Comparison of structure and interactions of models of **6.22** and **6.47** superimposed at the active site of TMPK*mt* (color codes: Mg - purple, H - white/gray, C - green, O - red, N - blue). Side chains of selected active site residues are shown in stick representations. Majority of hydrogen atoms were omitted for better clarity. Structures of the enzyme-inhibitor complexes were obtained by (semi)flexible docking and refinement of the model ligands into the crystal structure of TMPK*mt* co-crystallyzed with 5-CH_2OH dUMP (PDB entry code 1MRS).

It is rather unfortunate that enzyme assays indicated that combination of both modifications did not reinforce the TMPK*mt* binding affinity and led to very weak inhibitors. A possible explanation of the observed drop in the inhibitory potencies of the 5-CH_2OH substituted compounds may be related to an elevated steric strain in the bisubstituted analogs. A superposition of the models of inhibitors **6.22** and **6.47** at the active site of TMPK*mt* (Figure 6.2) shows that the 5′-tetrazolylmethyl moiety is firmly anchored in a polar cavity formed by the charged residues Asp9, Arg160, Asp163, and Glu166 and coordinated by the Mg^{2+} ion and a structural water molecule. However, the thymidine ring shows a higher degree of deviation between the two inhibitors, while the 5-CH_2OH group of **6.47** does not seem to reach up to an ideal stabilizing position.

As shown on Figure 6.2, the oxygen of the 5-CH_2OH group is oriented mainly by the interactions with the Arg74 and Phe70 residues into a position where it directs its H atom towards the hydrogen of C_β of Phe36 with a relatively short distance of only 1.85 Å (red line in Fig. 6.2). This leads to an electrostatic repulsion, which may be responsible for the observed weak inhibitory potency of **6.47**. In the crystal structure of TMPK*mt* co-crystallyzed with 5-CH_2OH dUMP the 5-hydroxymethyl group of dUMP is stabilized by hydrogen bonding interactions with the guanidine group of Arg74 and nitrogen atom of Pro37. Still, this does not exclude that a 2′,3′-α- fused cyclic sulfuryldiamide substituent may show synergistic inhibitory effects with a 5-CH_2OH group, since conformational restriction by a fused ring may very well change the topology of the molecular interactions with the enzyme.

6.3. Conclusions

In short, a small series of 5′-modified thymidine analogues was synthesized and evaluated as TMPK*mt* inhibitors. The analogues in which the 5′-hydroxyl group was replaced by an acetonitrile or a 5′-tetrazolylmethyl moiety proved capable of inhibiting the target enzyme with substantial affinity. Hence we developed a synthetic route that allowed combining these 5′-modifications with a hydroxymethyl moiety at

position 5 of the pyrimidine base. Given the recent interest in the phenomenon of hydroxymethylation of pyrimidine bases in mammalian genomes, the successful synthetic strategy, featuring pivaloyl protection of the 5-CH_2OH group prior to 5′-modification, may find future application in the synthesis of other 5-hydroxymethyl-2′-deoxyuridine tool compounds.

6.4. Experimental Section

6.4.1. Chemistry

All reagents were from standard commercial sources and of analytical grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. All reactions were carried out under argon atmosphere unless specified otherwise. A temperature of 25±5 °C is referred to as 'room temperature/ rt'. Precoated Merck silica-gel F254 plates were used for TLC; spots were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (40-60 µm, 60 Å). NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ), calibrated to the residual solvent signals or TMS. Exact mass measurements were performed on aWaters LCT PremierXETMTime of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1 v/v) mixture at 10 mL/min. The microwave reactions were carried out in Milestone MicroSYNTH Advanced Microwave Synthesis Labstation, equipped with 2 X 800 W magnetrons (effective maximum output 1500W pulsed/continuous), an optical fiber temperature sensor, a pressure sensor, under continues power mode in a closed PTFE vessel. NMR signals of sugar protons and carbons are indicated with a prime, and signals of base protons and carbons are given without a prime.

Diethyl ((N-methylsulfamoyl)methyl)phosphonate (6.8): To a solution of **6.7** (1.0 g, 9.1 mmol) in anhydrous THF (40.0 mL) at – 78 °C was added n-BuLi (1.6M, 11.5 mL,

18.3 mmol) dropwise under argon atmosphere. The mixture was stirred for an hour at – 78 °C and diethyl clorophosphate (0.67 mL, 4.6 mmol) was added slowly. The reaction mixture was stirred at 0 °C for an hour. The reaction was stopped by adding sat. NH_4Cl solution (10 mL) followed by extraction with CH_2Cl_2 (3 X 50 mL). The combined organic layers were dried over anhyd. Na_2SO_4. The desiccant was filtered off and the filtrate was concentrated. The residue was purified by flash column chromatography (50-80% EtOAc in hexanes) to afford unreacted starting material (450 mg) and product **6.8** as colourless oil (800 mg, 65 % based on recovered starting material). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.36 (td, *J* = 7.2, 0.9 Hz, 6H, $OCH_2C\mathit{H}_3$), 2.37 (d, *J* = 5.4 Hz, 3H, NCH_3), 3.60 (d, *J* = 16.2 Hz, 2H, SCH_2P), 4.22 (qt, *J* = 6.9 Hz, 0.9 Hz, 4H, $OC\mathit{H}_2CH_3$), 5.26 (q, *J* = 5.1 Hz, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δppm 16.14 (d, *J* = 6 Hz, $OCH_2\mathit{C}H_3$), 29.59 (NCH_3), 46.90 (d, *J* = 138.5 Hz, PCH_2S), 63.53 (d, *J* = 6.1 Hz, $O\mathit{C}H_2CH_3$). ^{31}P NMR (121 MHz, $CDCl_3$) δ ppm 13.52. ESI-HRMS for $[C_6H_{16}NO_5PS + H]^+$ calcd, 246.0565; found, 246.0548.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (6.11): Compound **6.10** (9.71 g, 20.64 mmoles) and anhydrous THF (106.4 mL) was placed in a Teflon flask under inert condition and cooled to 0 °C. In a separate polypropylene flask, anhydrous THF (79.8 mL) and anhydrous pyridine (31.9 mL) were placed under inert atomsphere; to this at 0 °C ~70%HF in pyridine (28.7 mL) was added drop wise. The chilled THF-Pyridine-HF.pyridine mixture was added dropwise to a flask containing compound **6.10** also at 0 °C and stirred at this temperature for 1 hour. The cold bath was removed and reaction continued at room temperature for 45 minutes. The reaction mixture was poured to an ice-cold solution of $NaHCO_3$ (100g in 500 mL) with vigorous stirring. The compound was extracted in EtOAc (3 X 150 mL), washed with brine and dried over anhyd.Na_2SO_4. The residue after evaporation was subjected to flash column chromatography (20 to 50% EtOAc in hexanes) to obtain compound **6.11** as white foam (2.615 g, 36 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.07 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $C(CH_3)_3$), 1.88 (s, 3H, 5-CH_3), 2.20 (ddd, *J* = 13.25, 6.37, 3.81 Hz, 1H, 2′-H), 2.32 (dt, *J* = 13.40, 6.63 Hz, 1H, 2′-H), 2.98 - 3.13 (m, 1H, 5′-OH), 3.68 - 3.80 (m, 1H,

5′-H), 3.85 - 3.96 (m, 2H, 5′ & 4′-H), 4.48 (dt, *J* = 6.44, 3.51 Hz, 1H, 3′-H), 6.16 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.35 - 7.48 (m, 1H, 6-H), 9.31 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.66 ($SiCH_3$), -4.50 ($SiCH_3$), 12.69 (5-CH_3), 18.15 ($C(CH_3)_3$), 25.92 ($C(CH_3)_3$), 40.76 (2′-C), 62.11 (5′-C), 71.81 (3′-C), 86.87 (1′-C), 87.84 (4′-C), 111.13 (5-C), 137.25 (6-C), 150.67 (2-C), 164.26 (4-C). ESI-HRMS for $[C_{16}H_{28}N_2O_5Si + H]^+$ calcd, 357.1846; found, 357.1847.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-((*E*)-2-(methylsulfonyl)vinyl) tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (6.13): To a solution of Dess-Martin reagent (1.28 g, 3 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C under argon atmosphere was added compound **6.11** (713 mg, 2 mmol) in CH_2Cl_2 (5 mL) and the reaction continued at room temperature. After 4 hours saturated $NaHCO_3$ solution (10 mL) was added and the product extracted in CH_2Cl_2 (3 X 20 mL). The organic layers were combined, washed with brine, dried over anhyd.Na_2SO_4, filtered. The filtrate was evaporated and dried under high vacuum to give aldehyde **6.12** as white foamy residue which was used without purification for Horner-Wittig reaction.

In a separate flask, Diethyl ((methylsulfonyl)methyl)phosphonate **6.5** (553 mg, 2.4 mmol) was dissolved in anhydrous THF (20 mL) under argon atmosphere. The mixture was brought to – 78 °C and n-BuLi (1.6M in hexanes, 1.38 mL, 2.2 mmol) was added slowly and stirred for 15 minutes. The solution of the above aldehyde **6.12** (2 mmol) in anhydrous THF (15 mL) was added dropwise. The flask was kept at – 78 °C for 1h and at room temperature overnight (18h). Water (10 mL) was added and the product extracted in EtOAc (3 X 50 mL). The combined organic layers were washed with brine, dried over anhyd.Na_2SO_4, filtered and filtrate was evaporated. The residue was purified by flash column chromatography (20-50 % EtOAc in hexanes) to afford **6.13** as white solid (175 mg, 20 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.03 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$), 0.84 (s, 9H, $C(CH_3)_3$), 1.89 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.16 - 2.24 (m, 2H, 2′-H), 2.91 (s, 3H, SO_2CH_3), 4.25 (dt, *J* = 6.37, 4.87 Hz, 1H, 3′-H), 4.33 - 4.43 (m, 1H, 4′-H), 6.22 (t, *J* = 6.74 Hz, 1H, 1′-H) 6.62 (dd, *J* = 15.08, 1.90 Hz, 1H, 6′-H), 6.93 (dd, *J* = 14.94, 4.10 Hz, 1H, 5′-H), 6.96 (d, *J* = 1.17 Hz, 1H, 6-H), 8.39 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.78 ($SiCH_3$), -4.66

($SiCH_3$), 12.64 (5-CH_3), 17.90 ($C(CH_3)_3$), 25.63 ($C(CH_3)_3$), 39.46 (2′-C), 42.68 (SO_2CH_3), 74.66 (3′-C), 84.05 (4′-C), 85.56 (1′-C), 111.93 (5-C), 130.75 (6′-C), 135.28 (6-C), 142.90 (5′-C), 150.00 (2-C), 163.14 (4-C). ESI-HRMS for $[C_{18}H_{30}N_2O_6SSi + H]^+$ calcd, 431.1672; found, 431.1519.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(2-(methylsulfonyl)ethyl)tetra hydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (6.14): To a solution of **6.13** (175 mg, 0.41 mmol) in EtOAc (10.0 mL) was added Pd-C (10% Pd, ~50% H_2O, 100 mg) and stirred with purging H_2 gas through the reaction mixture at room temperature for 4 hours. The catalyst was filtered over celite and filtrate was evaporated. The residue was dried under high vacuum to afford practically pure compound **6.14** (175 mg) which was used as such for the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.02 (s, 3H, $SiCH_3$), 0.03 (s, 3H, $SiCH_3$), 0.83 (s, 9H, $C(CH_3)_3$), 1.89 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 1.92 - 2.07 (m, 2H, 5′-H), 2.14 - 2.30 (m, 2H, 2′-H), 2.88 (s, 3H, SO_2CH_3), 3.04 (ddd, *J* = 13.7, 11.1, 5.7 Hz, 1H, 6′-H), 3.13 - 3.25 (m, 1H, 6′-H), 3.75 (dt, *J* = 10.40, 3.88 Hz, 1H, 4′-H), 4.08 - 4.12 (m, 1H, 3′-H), 6.09 (t, *J* = 6.88 Hz, 1H, 1′-H), 6.99 (d, *J* = 1.46 Hz, 1H, 6-H), 8.08 (br. s, 1H, NH). ESI-HRMS for $[C_{18}H_{32}N_2O_6SSi + H]^+$ calcd, 433.1829; found, 433.0574.

1-((2R,4S,5R)-4-hydroxy-5-(2-(methylsulfonyl)ethyl)tetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (6.15): To a solution of compound **6.14** (175 mg, 0.41 mmol) in anhydrous THF (3.0 mL) was added tetrabutylammonium fluoride (TBAF, 0.14 mL, 0.49 mmol) dropwise. The flask was heated to 40 °C overnight. The volatile materials were evaporated under high vacuum and the residue was purified by flash column chromatography (3% MeOH in CH_2Cl_2). The product was purified further by crystallization/ triturating in MeOH to afford compound **6.15** as a colorless needles (25 mg, 20 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 1.87 - 2.00 (m, 1H, 5′-H), 2.00 - 2.15 (m, 2H, 2′ & 5′-H), 2.25 (dt, *J* = 13.84, 6.99 Hz, 1H, 2′-H), 3.00 (s, 3H, SO_2CH_3), 3.19 (t, *J* = 8.05 Hz, 2H, 6′-H), 3.72 (dt, *J* = 8.57, 4.36 Hz, 1H, 4′-H), 4.08 - 4.18 (m, 1H, 3′-H), 5.34 (d, *J* = 4.39 Hz, 1H, 3′-OH), 6.15 (t, *J* = 7.03 Hz, 1H, 1′-H), 7.44 (d, *J* = 1.17 Hz, 1H, 6-H), 11.30 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.06 (5- CH_3), 25.78 (5′-C), 38.05 (2′-C),

40.15 (SO_2CH_3), 50.39 (6′-C), 72.84 (3′-C), 83.46 (1′-C), 83.83 (4′-C), 109.97 (5-C), 136.17 (6-C), 150.47 (2-C), 163.68 (4-C). ESI-HRMS for $[C_{12}H_{18}N_2O_6S + H]^+$ calcd, 319.0964; found 319.0963.

(*E*)-2-((2R,3S,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)-N-methylethenesulfonamide (6.16): Compound **6.11** (100 mg, 0.28 mmol)was reacted in similar fashion as described for **6.13** to get the aldehyde **6.12**. In a separate flask, to the solution of diethyl ((N-methylsulfamoyl)methyl)phosphonate **6.8** (83 mg, 0.36 mmol) in anhydrous THF (3 mL) at -78 °C under inert atmosphere was added n-BuLi (1.6M in hexanes, 0.39 mL, 0.62 mmol) drop wise and stirred for 1h. A solution of above aldehyde **6.12** (0.28 mmol) in THF (3 mL) was added and the mixture was kept at -78 °C for 1h then allowed to stir at room temperature overnight (18h). Water (5 mL) was added and the reaction mixture was extracted with EtOAc (3 X 25 mL). The combined organic layer was washed with brine, dried over anhyd.Na_2SO_4, filtered, and evaporated. The residue was purified by preperative TLC (50% EtOAc in toluene) to obtain **6.16** as a white foam (60 mg, 48 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.10 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.91 (s, 9H, $C(CH_3)_3$), 1.96 (d, *J* = 1.2 Hz, 3H, 5-CH_3), 2.23-2.29 (m, 2H, 2′-H), 2.76 (s, 3H, $NHCH_3$), 4.28-4.33 (m, 1H, 4′-H), 4.41 (td, *J* = 4.8, 1.2 Hz, 1H, 3′-H), 6.30 (t, *J* = 6.9Hz, 1H, 1′-H), 6.47 (dd, *J* = 15.3, 1.5 Hz, 1H, 6′-H), 6.82 (dd, *J* = 15.3, 4.5 Hz, 1H, 5′-H), 7.04 (d, *J* = 1.2 Hz, 1H, 6-H) ESI-HRMS for $[C_{18}H_{31}N_3O_6SSi + H]^+$ calcd, 446.1781; found, 446.1773.

2-((2R,3S,5R)-3-(hydroxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)-N-methylethanesulfonamide (6.17): To a solution of **6.16** (60 mg, 0.135 mmol) in 5:1 MeOH-THF (1.5 mL) at 0 °C was added NiCl.$6H_2O$ (16 mg, 0.075 mmol) and $NaBH_4$ (10.2 mg, 0.27 mmol). The reaction mixture was stirred for 1h at 0 °C and the solvent was evaporated. The residue suspended in EtOAc, filtrated over celite, the filtrate was evaporated. The residue obtained was dissolved in THF (1.5 mL) and was added TBAF (80 µL, 0.268 mmol). The reaction mixture was stirred at 40 °C for 1h. The solvent was evaporated to dryness and the residue was purified by flash column chromatography (3-7% MeOH in CH_2Cl_2) to afford **6.17** (5

mg, 12 %) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, *J* = 0.88 Hz, 3H, 5-CH_3), 1.91 (td, *J* = 9.15, 4.54 Hz, 1H, 5′-H), 1.96 - 2.10 (m, 2H, 2′-H and 5′-H), 2.24 (dt, *J* = 13.84, 6.99 Hz, 1H, 2′-H), 2.57 (d, *J* = 4.98 Hz, 3H, N-CH_3), 3.07 (dt, *J* = 9.81, 5.64 Hz, 2H, 6′-H), 3.73 (dt, *J* = 8.42, 4.43 Hz, 1H, 4′-H), 4.11 (dd, *J* = 6.74, 4.10 Hz, 1H, 3′-H), 5.34 (d, *J* = 4.39 Hz, 1H, 3′-OH), 6.14 (t, *J* = 6.88 Hz, 1H, 1′-H), 6.92 (q, *J* = 4.98 Hz, 1H, CH_3NH) 7.42 (d, *J* = 1.17 Hz, 1H, 6-H), 11.30 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 11.96 (5-CH_3), 27.01 (5′-C), 28.47 (NCH_3), 38.04 (2′-C), 46.01 (6′-C), 72.73 (3′-C), 83.29 (1′-C), 83.62 (4′-C), 109.84 (5-C), 136.00 (6-C), 150.33 (2-C), 163. 55 (4-C). ESI-HRMS for $[C_{12}H_{19}N_3O_6S + H]^+$ calcd, 334.1073; found, 334.1085.

(*E*)-3-((2R,3S,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)acrylonitrile (6.18): Compound **6.11** (1.22 g, 3.43 mmol) was reacted in similar fashion as described for **6.13** to get the aldehyde **6.12**. In a separate flask, to a solution of cyanomethyltriphenylphosphonium chloride (3.48 g, 10.3 mmol) in anhydrous THF (35 mL) at 0 °C under argon atmosphere was added drop wise n-BuLi (1.6M in hexanes, 6.4 mL, 10.3 mmol) and stirred for 30 minutes. To this ylide at 0 °C was added slowly a solution of above aldehyde **6.12** (3.43 mmol) in anhydrous THF (15 mL) and stirred at room temperature overnight. The reaction was quenched with water (30 mL) and extracted with EtOAc (3 X 100 mL). Combined organic layer was washed with brine, dried over anhyd.Na_2SO_4, filtered and evaporated. The residue obtained was purified by flash column chromatography (15-30% EtOAc in hexanes) to afford **6.18** as a light yellow foam (740.4 mg, 57 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $C(CH_3)_3$), 1.96 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 2.27 - 2.36 (m, 2H, 2′-H), 4.27 - 4.35 (m, 2H, 3′-H and 4′-H), 5.67 (dd, *J* = 16.26, 1.61 Hz, 1H, 6′-H), 6.19 (t, *J* = 6.59 Hz, 1H, 1′-H), 6.80 (dd, *J* = 16.11, 4.69 Hz, 1H, 5′-H), 7.01 (d, *J* = 1.17 Hz, 1H, 6-H), 9.46 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.82 ($SiCH_3$), -4.63 ($SiCH_3$), 12.65 (5-CH_3), 17.89 ($C(CH_3)_3$), 25.63 ($C(CH_3)_3$), 39.79 (2′-C) 74.69 (3′-C), 84.96 (4′-C), 86.14 (1′-C), 101.00 (6′-C), 111.74 (5-C), 116.56 (7′-C), 135.74 (6-C) 149.95 (5′-C), 150.25 (2-C), 163.80 (4-C). ESI-HRMS for $[C_{18}H_{27}N_3O_4Si + H]^+$ calcd, 378.1849; found, 378.1852.

3-((2R,3S,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)propanenitrile (6.19): To a solution of 6.18 (443 mg, 1.18 mmol) in 3:1 anhydrous pyridine-MeOH (2 mL) was added $NaBH_4$ (45 mg, 1.18 mmol) and the reaction mixture was stirred at 120 °C under inert atmosphere for 4h. The reaction mixture was evaporated and the residue was purified by flash column chromatography (30-50% EtOAc in hexanes) to afford 6.19 as white foam (240 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $C(CH_3)_3$), 1.84 - 1.93 (m, 1H, 5′-H), 1.95 (d, *J* = 1.46 Hz, 3H, 5-CH_3), 2.02 - 2.16 (m, 1H, 5′-H), 2.27 (t, *J* = 6.44 Hz, 2H, 2′-H), 2.41 - 2.62 (m, 2H, 6′-H), 3.80 (ddd, *J* = 9.30, 5.35, 3.81 Hz, 1H, 4′-H), 4.12 - 4.20 (m, 1H, 3′-H), 6.08 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.05 (d, *J* = 1.17 Hz, 1H, 6-H), 8.44 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.81 ($SiCH_3$), -4.53 ($SiCH_3$), 12.62 (5-CH_3), 14.22 (6′-C), 17.91 ($C(CH_3)_3$), 25.68 ($C(CH_3)_3$), 28.94 (5′-C), 40.15 (2′-C), 74.43 (3′-C), 84.04 (4′-C), 85.60 (1′-C), 111.35 (5-C), 119.11 (7′-C), 135.87 (6-C), 149.86 (2-C), 163.32 (6-C). ESI-HRMS for $[C_{18}H_{29}N_3O_4Si + H]^+$ calcd, 380.2006; found, 380.2015.

3-((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)propanenitrile (6.20): To the stirring solution of compound **6.19** (100 mg, 0.26 mmol) in anhydrous THF (2 mL) was added tetrabutylammonium fluoride (TBAF, 1M in THF, 0.5 mL, 0.5 mmol) under inert atmosphere and stirred at room temperature for 4h. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (EtOAc) to afford product **6.20** as a white solid (53 mg, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 1.83 - 1.98 (m, 2H, 5′-H), 2.05 (ddd, *J* = 13.55, 6.52, 3.95 Hz, 1H, 2′-H), 2.22 (dt, *J* = 13.77, 6.88 Hz, 1H, 2′-H), 2.52 - 2.62 (m, 2H, 6′-H), 3.63 - 3.74 (m, 1H, 4′-H), 4.04 - 4.17 (m, 1H, 5′-H), 5.34 (d, *J* = 4.39 Hz, 1H, 3′-OH), 6.15 (t, *J* = 6.88 Hz, 1H, 1′-H), 7.44 (d, *J* = 1.17 Hz, 1H, 6-H), 11.30 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 11.96 (5-CH_3), 13.20 (6′-C), 28.37 (5′-C), 38.01 (2′-C), 72.50 (3′-C), 83.42 (1′-C), 83.92 (4′-C), 109.75 (5-C), 120.30 (7′-C), 136.13 (6-C), 150.33 (2-C), 163.56 (4-C). ESI-HRMS for $[C_{12}H_{15}N_3O_4 - H]^-$ calcd, 264.099; found, 264.0617.

1-((2R,4S,5R)-5-(2-(1H-tetrazol-5-yl)ethyl)-4-((*tert*-butyldimethylsilyl)oxy)tetra hydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (6.21): Compound **6.19** (120 mg, 0.32 mmol) was dessolved in anhydrous toluene (5 mL), to this was added dibutyltin oxide (Bu_2SnO, 16 mg) and azidotrimethylsilane ($TMSN_3$, 158 µL, 1.6 mmol) under argon atmosphere. The reaction vessel was sealed with septum and stirred at 110 °C for 4h. The volatiles were evaporated under reduced pressure and the residue was purified by flash column chromatography (2% AcOH/ EtOAc) to afford **6.21** as a white foam (43 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.00 (s, 6H, $Si(CH_3)_2$), 0.78 (s, 9H, $C(CH_3)_3$), 1.73 (d, J = 0.88 Hz, 3H, 5-CH_3), 1.86 - 2.07 (m, 3H, 2′-H and 5′-H), 2.21 (dt, J = 13.62, 6.96 Hz, 1H, 2′-H), 2.80 - 2.96 (m, 2H, 6′-H), 3.61 (dt, J = 8.42, 4.43 Hz, 1H, 4′-H), 4.20 (dt, J = 6.59, 4.03 Hz, 1H, 3′-H), 6.04 (t, J = 6.88 Hz, 1H, 1′-H), 7.33 (d, J = 1.17 Hz, 1H, 6-H), 11.23 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm -4.89 ($SiCH_3$), -4.72 ($SiCH_3$), 12.10 (5-CH_3), 17.61 ($C(CH_3)_3$), 19.63 (6′-C), 25.65 ($C(CH_3)_3$), 30.42 (5′-C), 38.60 (2′-C), 74.35 (3′-C), 83.46 (1′-C), 84.44 (4′-C), 109.89 (5-C), 136.21 (6-C), 150.39 (2-C), 155.69 (7′-C) 163.66 (4-C). ESI-HRMS for $[C_{18}H_{30}N_6O_4Si - H]^-$ calcd, 421.2020; found, 421.1249.

1-((2R,4S,5R)-5-(2-(1H-tetrazol-5-yl)ethyl)-4-hydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (6.22): Compound **6.21** (40 mg, 0.1 mmol) was dissolved in anhydrous THF (2 mL) and added TBAF (0.5 mL, 0.5 mmol) at room temperature. After 4h, MeOH (2 mL) was added, followed by $CaCO_3$ (1g) with vigorous stirring. After 10 minutes was added Dowex resin (H^+ form, 1g) portion wise. The mixture was stirred for 30 minutes and filtered over a pad of celite. The filtrate was concentrated and purified by flash column chromatography (5-8 % MeOH + 2% AcOH in CH_2Cl_2) to afford title compound **6.22** as a white solid (10 mg, 34%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.80 (d, J = 1.17 Hz, 3H, 5-CH_3), 1.89 - 2.14 (m, 3H, 2′-H and 5′-H), 2.14 - 2.33 (m, 1H, 2′-H), 2.87 - 3.06 (m, 2H, 6′-H), 3.66 (dt, J = 8.57, 4.36 Hz, 1H, 4′-H), 4.05 - 4.17 (m, 1H, 3′-H), 5.30 (br. s, 1H, 3′-OH), 6.14 (t, J = 6.88 Hz, 1H, 1′-H), 7.39 (d, J = 0.88 Hz, 1H, 6-H), 11.30 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 11.99 (5-CH_3), 19.39 (6′-C), 30.56 (5′-C), 38.20 (2′-C), 72.73 (3′-C), 83.18 (1′-C), 84.30 (4′-C), 109.79 (5-C), 135.94 (6-C), 150.33 (2-C), 155.07

(7′-C), 163.56 (4-C). ESI-HRMS for $[C_{12}H_{16}N_6O_4 - H]^-$ calcd, 307.1155; found, 307.0597.

6′-(*N*-hydroxyformamidine)-β-D-thymidine (6.23): Compound **6.20** (30 mg, 0.11 mmol) was dissolved in ethanol (2 mL) to this was added hydroxylamine solution (83 µL) and stirred at room temperature for 2 days. The volatiles were evaporated under high vacuum and the residue purified by column chromatography using 5-10% MeOH in CH_2CL_2 with 0.1% TEA to afford title compound **6.23** (28 mg, 83%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.64 - 1.75 (m, 1H, 5′-H), 1.78 (d, *J* = 1.17 Hz, 3H, 5-CH_3), 1.81 - 1.89 (m, 1H, 5′-H), 1.91 - 2.09 (m, 3H, 2X6′-H, 1X2′-H), 2.10-2.22 (m, 1H, 2′-H), 3.56 - 3.72 (m, 1H, 4′-H), 3.95 - 4.14 (m, 1H, 3′-H), 5.24 (br.s, 1H, 3′-OH), 5.34 (s, 2H, NH_2) 6.10 (t, *J* = 6.88 Hz, 1H, 1′-H), 7.37 (d, *J* = 1.17 Hz, 1H, 6-H), 8.71 (br.s, 1H, NOH), 11.26 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.05 (5-CH_3), 27.30 (6′-C), 30.19 (5′-C), 38.37 (2′-C), 72.84 (3′-C), 83.11 (1′-C), 85.17 (4′-C), 109.70 (5-C), 135.85 (6-C), 150.35 (2-C), 152.22 (7′-C), 163.57 (4-C). ESI-HRMS $[C_{12}H_{18}N_4O_5 + H]^+$, calcd, 299.1355; found, 299.1350.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (6.26): Removal of primary TBDMS group using the procedure described for compound **6.11**, compound **6.25** (1.8 g, 4 mmol) rendered **6.26** (600 mg, 53%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 2.24 - 2.32 (m, 2H, 2′-H) 2.50 (br. s, 1H, 5′-OH), 3.69 - 3.82 (m, 1H, 5′-H), 3.87 - 4.00 (m, 2H, 4′-H and 5′-H), 4.43 - 4.55 (m, 1H, 3′-H), 5.74 (dd, *J* = 8.20, 2.05 Hz, 1H, 5-H), 6.18 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.65 (d, *J* = 8.20 Hz, 1H, 6-H), 8.89 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.64 ($SiCH_3$), -4.47 ($SiCH_3$), 18.18 ($C(CH_3)_3$), 25.93 ($C(CH_3)_3$), 41.09 (2′-C), 62.09 (5′-C), 71.64 (3′-C), 87.02 (1′-C), 87.82 (4′-C), 102.71 (5-C), 141.31 (6-C), 150.42 (2-C), 163.40 (4-C). ESI-HRMS for $[C_{15}H_{26}N_2O_5Si + H]^+$ calcd, 343.1689; found, 343.1682.

(*E*)-3-((2R,3S,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)acrylonitrile (6.27): Following

the oxidation and Wittig reaction procedure described for compound **6.18**, compound **6.26** (600 mg, 1.75 mmol) rendered **6.27** (325 mg, 51%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.10 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 2.19 - 2.43 (m, 2H, 2′-H), 4.24 - 4.30 (m, 1H, 3′-H), 4.30 - 4.37 (m, 1H, 4′-H), 5.67 (dd, *J* = 16.40, 1.76 Hz, 1H, 6′-H), 5.81 (dd, *J* = 8.20, 2.34 Hz, 1H, 5-H), 6.19 (t, *J* = 6.44 Hz, 1H, 1′-H), 6.77 (dd, *J* = 16.11, 4.69 Hz, 1H, 5′-H), 7.21 (d, *J* = 8.20 Hz, 1H, 6-H), 8.65 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.82 ($SiCH_3$), -4.63 ($SiCH_3$), 17.90 ($C(CH_3)_3$), 25.63 ($C(CH_3)_3$), 40.06 (2′-C), 74.59 (3′-C), 85.04 (4′-C), 86.30 (1′-C), 101.19 (6′-C), 103.21 (5-C), 116.42 (7′-C), 139.83 (6-C), 149.61 (5′-C), 149.82 (2-C), 162.71 (4-C). ESI-HRMS for $[C_{17}H_{25}N_3O_4Si + CH_3CN + H]^+$ calcd, 405.1958; found, 405.1955.

3-((2R,3S,5R)-3-((*tert*-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)propanenitrile (6.28): Following the reaction procedure described for compound **6.14** but using MeOH as solvent, compound **6.27** (55 mg, 0.15 mmol) gave **6.28** as a white solid (50 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (d, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 1.89 (dddd, *J* = 13.84, 9.45, 7.47, 6.15 Hz, 1H, 5′-H), 2.03 - 2.15 (m, 1H, 5′-H), 2.19 - 2.38 (m, 2H, 2′-H), 2.42 - 2.62 (m, 2H, 6′-H), 3.82 (ddd, *J* = 9.37, 5.42, 3.66 Hz, 1H, 4′-H), 4.14 (dt, *J* = 7.03, 5.42 Hz, 1H, 3′-H), 5.77 (d, *J* = 7.91 Hz, 1H, 5-H), 6.08 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.26 (d, *J* = 8.20 Hz, 1H, 6-H), 8.40 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.83 ($SiCH_3$), -4.54 ($SiCH_3$), 14.25 (6′-C), 17.90 ($C(CH_3)_3$), 25.67 ($C(CH_3)_3$), 28.98 (5′-C), 40.40 (2′-C), 74.37 (3′-C), 84.20 (4′-C), 85.95 (1′-C), 102.83 (5-C), 119.01 (7′-C), 140.08 (6-C), 149.84 (2-C), 162.84 (4-C). ESI-HRMS for $[C_{17}H_{27}N_3O_4Si + H]^+$ calcd, 366.1849; found, 366.1842.

3-((2R,3S,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxytetrahydrofuran-2-yl)propanenitrile (6.29): Following the reaction procedure described for compound **6.20**, compound **6.28** (50 mg, 0.14 mmol) rendered **6.29** (25 mg, 73%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.89 - 2.14 (m, 2H, 5′-H), 2.20 - 2.37 (m, 2H, 2′-H), 2.48 - 2.69 (m, 2H, 6′-H), 3.85 (dt, *J* = 9.23, 4.47 Hz, 1H, 4′-H), 4.13 - 4.25 (m, 1H, 3′-H), 5.71 (d, *J* = 7.91 Hz, 1H, 5-H), 6.18 (t, *J* = 6.74 Hz, 1H, 1′-

H), 7.62 (d, *J* = 8.20 Hz, 1H, 6-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 14.48 (6′-C), 30.32 (5′-C), 40.21 (2′-C), 74.68 (3′-C), 85.87 (4′-C), 86.71 (1′-C), 103.04 (5-C), 120.79 (7′-C), 142.57 (6-C), 152.07 (2-C), 166.15 (4-C). ESI-HRMS for $[C_{11}H_{13}N_3O_4 - H]^-$ calcd, 250.0828; found, 250.0833.

(1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl acetate (6.32): Following the procedure described for the synthesis of **6.11**, compound **6.31** (1 g, 1.94 mmol) rendered **6.32** (265 mg, 33%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 2.06 (s, 3H, OAc), 2.18 - 2.40 (m, 2H, 2′-H), 3.77 (dd, *J* = 12.7, 3.2 Hz,1H, 5′-H), 3.88 - 4.04 (m, 2H, 5′ & 4′-H), 4.52 (dt, *J* = 5.80, 3.70 Hz, 1H, 3′-H), 4.79 - 4.97 (m, 2H, 5-CH_2OH), 6.24 (t, *J* = 6.49 Hz, 1H, 1′-H), 8.11 (s, 1H, 6-H), 9.02 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.90, -4.74 ($SiCH_3$), 17.94 ($C(CH_3)_3$), 21.14 (Ac-C), 25.69 ($C(CH_3)_3$), 41.36 (1′-C), 58.82 (5-CH_2OH), 61.82 (5′-C), 71.66 (3′-C), 86.49 (1′-C), 88.12 (4′-C), 109.02 (5-C), 142.86 (6-C), 149.94 (2-C), 162.58 (4-C), 171.96 (Ac-C= O). ESI-HRMS for $[C_{18}H_{30}N_2O_7Si + H]^+$ calcd, 415.1901; found, 415.1904.

5-(((benzyloxy)methoxy)methyl)-3-((benzyloxy)methyl)-1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (6.34): To a mixture of compound **6.33** (150 mg, 0.31 mmol) and anhydrous diisopropylethylamine (DIPEA, 160 µL, 0.92 mmol) in anhydrous DMF (2.5 mL) at 0 °C under inert atmosphere was added drop wise benzyloxymethyl chloride (~75 % BOMCl, 94 µL, 0.68 mmol) and stirred at room temperature overnight. Saturated aq.NH_4Cl (5 mL) was added to quench the reaction. The products were extracted in EtOAc (50 mL), organic layer washed with water, brine, dried over anhyd.Na_2SO_4 and evaporated. The residue was purified by flash column chromatography (5-10 % EtOAc in hexanes) to yield **6.34** (80 mg, 36 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08 - 0.12 (m, 12H, $2XSi(CH_3)_2$), 0.88 - 0.94 (m, 18H, $2XC(CH_3)_3$), 1.97 (ddd, *J* = 13.34, 7.64, 5.97 Hz, 1H, 2′-H), 2.30 (ddd, *J* = 13.16, 5.74, 2.44 Hz, 1H, 2′-H), 3.76 (dd, *J* = 11.40, 3.08 Hz, 1H, 5′-H), 3.82 (dd, *J* = 11.22, 3.26 Hz, 1H, 5′-H), 3.96 (q, *J* = 3.08 Hz, 1H, 4′-H), 4.34 - 4.47 (m, 3H, 3′-H & 5-CH_2),

4.65 (s, 2H, OCH_2Ph), 4.71 (s, 2H, OCH_2Ph), 4.80 - 4.87 (m, 2H, OCH_2O), 5.50 (s, 2H, NCH_2O), 6.31 (dd, *J* = 7.87, 5.70 Hz, 1H, 1′-H), 7.22 - 7.41 (m, 10H, CH_2*Ph*), 7.65 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.50, -5.40, -4.84, -4.65 ($SiCH_3$), 17.98, 18.39 (*C*$(CH_3)_3$), 25.73, 25.93 (C(*C*$H_3)_3$), 41.35 (1′-C), 62.99 (5-CH_2), 63.07 (5′-C), 69.39 (*C*H_2Ph), 70.45 (NCH_2O), 72.24 (3′-C), 72.28 (*C*H_2Ph), 85.95 (1′-C), 87.94 (4′-C), 94.36 (OCH_2O), 110.72 (5-C) 126.97, 127.61, 127.69, 127.92, 128.26, 128.39, 128.56 (CH_2*Ph*), 136.92 (6-C), 137.70, 137.93 (CH_2*Ph*), 150.80 (2-C), 162.24 (4-C). ESI-HRMS for $[C_{38}H_{58}N_2O_8Si_2 + H]^+$ calcd, 727.381; found, 727.3805.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy) methyl)tetrahydrofuran-2-yl)-5-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)-3-((2-(trimethylsilyl)ethoxy)methyl)pyrimidine-2,4(1H,3H)-dione (6.35): Under an inert condition compound **6.33** (486 mg, 1 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL) and anhydrous DIPEA (0.5 mL, 3 mmol) and added ethyltrimethysilylchloromethyl ether (SEMCl, 0.44 ml, 2.5 mmol). The reaction was continued at 40 °C for 6h. The reaction mixture was partitioned between brine (5 mL) and EtOAc (20 mL). The organic layer was separated and dried over anhyd.Na_2SO_4. The residue after evaporation of organic phase was purified by flash column chromatography (5% EtOAc in hexanes) to afford compound **6.35** (370 mg, 50 %) as a glass. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (s, 9H, $Si(CH_3)_3$), 0.03 (s, 9H, $Si(CH_3)_3$), 0.08 (s, 3H, $Si(CH_3)_2$), 0.09 (s, 3H, $Si(CH_3)_2$), 0.11 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 0.94-1.02 (m, 4H, $(CH_3)_3SiCH_2$), 2.00 (ddd, *J* = 15.3 Hz,7.8 Hz, 1.8 Hz, 1H, 2′-H), 2.31 (ddd, *J* = 13.2 Hz, 5.7 Hz, 2.4 Hz, 1H, 2′-H), 3.60-3.73 (m, 4H, OC*H*$_2$$CH_2$ $(SiCH_3)_3$), 3.76 (dd, *J* = 11.10, 3.15 Hz, 1H, 5′-H), 3.82 (dd, *J* = 11.29, 3.33 Hz, 1H, 5′-H), 3.96 (q, *J* = 2.96 Hz, 1H, 4′-H), 4.31 (d, *J* = 12.24 Hz, 1H, 5-CH_2), 4.37 (d, *J* = 12.0 Hz, 1H, 5-CH_2), 4.37-4.42 (m, 1H, 3′-H), 4.74 (s, 2H, OCH_2O), 5.40 (s, 2H, NCH_2O), 6.32 (1H, dd, *J* = 7.8 Hz, 5.7 Hz, 1′-H), 7.65 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm -5.50, -5.38, -4.84, -4.67 ($Si(CH_3)_2$), -1.44, -1.38 ($Si(CH_3)_3$), 17.97 (t-Bu-tC), 18.07, 18.11 (SEM $SiCH_2$); 18.40 (*C*$(CH_3)_3$), 25.72, 25.95 (C(*C*$H_3)_3$), 41.34 (2′-C), 62.96 (5-CH_2O), 62.99 (5′-C), 65.28, 67.49 (SEM CH_2O), 70.22 (NCH_2O), 72.43 (3′-C), 86.11 (1′-C), 88.06 (4′-C), 94.94

(OCH_2O), 111.08 (5-C), 136.71 (6-C), 150.99 (2-C), 162.34 (4-C). ESI-HRMS for $[C_{34}H_{70}N_2O_8Si_4 + H]^+$ calcd, 747.4287; found, 747.4144.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran -2-yl)-5-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)-3-((2-(trimethylsilyl)ethoxy) methyl)pyrimidine-2,4(1H,3H)-dione (6.36): Following the procedure described for the synthesis of **6.11**, compound **6.35** (350 mg, 0.48 mmol) gave compound **6.36** as a glassy wax (114 mg, 38 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (s, 9H, $Si(CH_3)_3$), 0.03 (s, 9H, $Si(CH_3)_3$), 0.09 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$); 0.92-1.00 (m, 4H, SEM SiCH_2), 2.22-2.41 (m, 2H, 2′-H), 2.58 (t, *J* = 4.8 Hz, 1H, 5′-OH), 3.60-3.72 (m, 4H, SEM CH_2O), 3.72-3.80 (m, 1H, 5′-H), 3.87-3.93 (m, 1H, 5′-H), 3.93-3.98 (m, 1H, 4′-H), 4.39 (s, 2H, 5-CH_2O), 4.51 (1H, dt, *J* = 6.6, 3.81 Hz, 3′-H), 4.73 (s, 2H, OCH_2O), 5.39 (s, 2H, NCH_2O), 6.20 (t, *J* = 6.44 Hz, 1H, 1′-H), 7.75 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm –4.86, -4.69 ($Si(CH_3)_2$), -1.43, -1.42 ($Si(CH_3)_3$), 17.95 ($C(CH_3)_3$), 18.12 (SEM $SiCH_2$), 25.71 ($C(CH_3)_3$), 40.91 (2′-C), 62.04 (5′-C), 62.27 (5-CH_2O), 65.56, 67.58 (SEM CH_2O), 70.03 (NCH_2O-C), 71.68 (3′-C), 87.80 (1′-C), 87.88 (4′-C), 93.96 (OCH_2O-C), 110.24 (5-C), 138.05 (6-C), 150.86 (2-C), 162.03 (4-C). ESI-HRMS for $[C_{28}H_{56}N_2O_8Si_3 + H]^+$ calcd, 633.3423; found, 633.3451.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-((*E*)-2-(methylsulfonyl)vinyl) tetrahydrofuran-2-yl)-5-(((2-(trimethylsilyl)ethoxy)methoxy)methyl)-3-((2-(trimethylsilyl)ethoxy) methyl)pyrimidine-2,4(1H,3H)-dione (6.37): Following the procedure described for the synthesis of **6.13**, compound **6.36** (113 mg, 0.18 mmol) rendered **6.37** as white solid (33 mg, 29 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.00 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$), 0.10 (s, 3H, $Si(CH_3)_2$), 0.10 (s, 3H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 0.92-1.02 (s, 4H, SEM SiCH_2), 2.15-2.40 (m, 2H, 2′-C), 2.97 (s, 3H, SO_2CH_3), 3.58-3.75 (m, 4H, SEM CH_2O), 4.33 (dt, *J* = 6.4, 4.7 Hz, 1H, 4′-H), 4.40 (s, 2H, 5-CH_2O), 4.47 (td, *J* = 4.7, 1.8 Hz, 1H, 3′-H), 4.73 (s, 2H, OCH_2O), 5.39 (s, 2H, NCH_2O), 6.33 (t, *J* = 6.6 Hz, 1H, 1′-H), 6.69 (dd, *J* = 15.3 Hz, 1.8 Hz, 1H, 6′-H), 7.00 (1H, dd, *J* = 15 Hz, 4.2 Hz, 5′-H), 7.34 (s, 1H, 6-H). ESI-HRMS for $[C_{30}H_{58}N_2O_9SSi_3 + Na]^+$ calcd, 729.3069; found, 729.1077.

1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy) methyl)tetrahydrofuran-2-yl)-3-(4-methoxybenzyl)-5-(((4-methoxybenzyl)oxy) methyl)pyrimidine-2,4(1H,3H)-dione (6.38): To a stirring solution of **6.33** (200 mg, 0.41 mmol) in anhydrous DMF (2 mL) at 0 °C under argon atmosphere was added 4-methoxybenzyl chloride (PMBCl, 167 µL, 1.23 mmol) followed by NaH (60% in mineral oil, 50 mg, 1.23 mmol) portion wise and stirred at room temperature for 4h. EtOAc (20 mL) and saturated aq.NH_4Cl (10 mL) was added. The aqueous layer was extracted with EtOAc (3 X 20 mL) and the combined organic layers were dried over anhyd.Na_2SO_4, filtered, evaporated. The residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford the title compound **6.38** as a white foam (40 mg, 13%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.05 (s, 3H, $SiCH_3$), 0.06 (s, 6H, $Si(CH_3)_3$), 0.07 (s, 3H, $SiCH_3$), 0.88 (s, 9H, $C(CH_3)_3$), 0.89 (s, 9H, $C(CH_3)_3$), 1.97 (ddd, *J* = 13.40, 7.69, 6.15 Hz, 1H, 2′-H), 2.26 (ddd, *J* = 13.18, 5.71, 2.49 Hz, 1H, 2′-H), 3.72 - 3.76 (m, 1H, 5′-H), 3.77 (s, 3H, PMB OCH_3), 3.79 (s, 2H, PMB OCH_3), 3.92 (q, *J* = 3.22 Hz, 1H, 5′-H), 4.18 - 4.33 (m, 2H, 5-CH_2O,) 4.37 (dt, *J* = 5.64, 2.60 Hz, 1H, 3′-H), 4.52 (s, 2H, PMB OCH_2), 4.95 - 5.13 (m, 2H, PMB NCH_2), 6.32 (dd, *J* = 7.91, 5.56 Hz, 1H, 1′-H), 6.82 (d, *J* = 8.79 Hz, 2H, Ar), 6.86 (d, *J* = 8.49 Hz, 2H, Ar), 7.26 (d, *J* = 8.49 Hz, 2H, Ar), 7.44 (d, *J* = 8.79 Hz, 2H, Ar), 7.59 (s, 1H, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.51, -5.43, -4.84, -4.66 ($SiCH_3$), 17.98, 18.39 ($C(CH_3)_3$), 25.74, 25.94 ($C(CH_3)_3$), 41.17 (2′-C), 43.88 (NCH_2-C), 55.23, 55.25 (OCH_3), 63.01 (5′-C), 65.06 (5-CH_2O), 72.30 (3′-C), 72.81 (PMB CH_2O), 85.83 (1′-C), 87.80 (4′-C), 111.19 (5-C), 113.69, 113.80, 129.05, 129.51, 130.06, 130.73 (Ar-C) 135.99 (6-C), 150.80 (2-C), 159.04, 159.27 (Ar-C), 162.23 (2-C). ESI-HRMS for $[C_{38}H_{58}N_2O_8Si_2 + H]^+$ calcd, 727.3810; found, 727.3797.

(1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy) methyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.39): To a solution of **6.33** (2.0 g, 4.1 mmol) in anhydrous pyridine (20 mL) and DMAP (100 mg) under inert atmosphere was added pivaloyl chloride (0.76 mL, 6.16 mmol) and the reaction mixture was stirred at room temperature overnight (18h). Pyridine was evaporated under reduced pressure and the residue was purified by flash

column chromatography (10-25 % EtOAc in hexanes) to afford **6.39** as a white solid (2.0g, 74 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, $SiCH_3$), 0.09 (s, 3H, $SiCH_3$), 0.10 (s, 6H, $Si(CH_3)_2$), 0.90 (d, 9H, $C(CH_3)_3$), 0.91 (d, 9H, $C(CH_3)_3$), 1.19 (s, 9H, Piv $C(CH_3)_3$), 2.01 (ddd, *J* = 13.40, 7.69, 5.86 Hz, 1H, 2′-H), 2.32 (ddd, *J* = 13.18, 5.86, 2.64 Hz, 1H, 2′-H), 3.73 - 3.85 (m, 2H, 5′-H), 3.96 (q, *J* = 3.22 Hz, 1H, 4′-H), 4.40 (dt, *J* = 5.49, 2.67 Hz, 1H, 3′-H), 4.80 (s, 2H, 5-CH_2), 6.28 (dd, *J* = 7.91, 5.86 Hz, 1H, 1′-H), 7.78 (s, 1H, 6-H), 8.28 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -5.42 ($SiCH_3$), -5.38 ($SiCH_3$), -4.83 ($SiCH_3$), -4.65 ($SiCH_3$), 18.01 ($C(CH_3)_3$), 18.42 ($C(CH_3)_3$), 25.74 ($C(CH_3)_3$), 25.93 ($C(CH_3)_3$), 27.15 (Piv $C(CH_3)_3$), 38.79 (Piv $C(CH_3)_3$), 41.39 (2′-C), 59.07 (5-CH_2-C), 63.11 (5′-C), 72.36 (3′-C), 85.51 (1′-C), 88.09 (4′-C), 109.62 (5-C), 140.25 (6-C), 149.76 (2-C), 161.86 (4-C), 178.23 (Piv CO). ESI-HRMS for $[C_{27}H_{50}N_2O_7Si_2 + H]^+$ calcd, 571.3235; found, 471.3242.

(1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydro furan-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.40): Following the procedure described for the synthesis of **6.11**, compound **6.39** (2.0 g, 3.05 mmol) gave compound **6.40** as a white solid (822 mg, 59 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 1.18 (s, 9H, Piv $C(CH_3)_3$), 2.19 - 2.38 (m, 2H, 2′-H), 2.94 (t, *J* = 5.32 Hz, 1H, 5′-OH), 3.70 - 3.81 (m, 1H, 5′-H), 3.90 - 3.99 (m, 2H, 5′ & 4′-H), 4.52 (dt, *J* = 6.25, 3.54 Hz, 1H, 3′-H), 4.83 - 4.94 (m, 2H, 5-CH_2O), 6.23 (t, *J* = 6.67 Hz, 1H, 1′-H), 8.08 (s, 1H, 6-H), 8.78 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.86, -4.70 ($SiCH_3$), 17.98 ($C(CH_3)_3$), 25.73 ($C(CH_3)_3$), 27.04 ($C(CH_3)_3$), 38.97 (piv $C(CH_3)_3$), 41.42 (2′-C), 58.63 (5-CH_2O-C), 62.06 (5′-C), 71.91 (3′-C), 86.60 (1′-C), 88.29 (4′-C), 109.26 (5-C), 142.71 (6-C), 149.70 (2-C), 161.95 (4-C), 179.70 (Piv CO). ESI-HRMS for $[C_{21}H_{36}N_2O_7Si + H]^+$ calcd, 457.2370; found, 457.2414.

(1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(2-cyanovinyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.41): Following the similar procedure to synthesize **6.12**, the reaction of compound **6.40** (0.8 g, 1.75 mmol), Dess-Martin reagent (0.48 M in CH_2Cl_2, 4.4 mL, 2.1 mmol) at room temperature for 7h gave aldehyde as white foamy residue.

In a separate flask, to a solution of cyanomethyltriphenylphosphonium chloride (1.78 g, 5.25 mmol) in anhydrous THF (30 mL) at -78 °C under argon condition was added drop wise n-BuLi (1.6M in hexanes, 3.3 mL, 5.25 mmol) and stirred for 30 minutes. To this ylide at -78 °C was added slowly a solution of above aldehyde in anhydrous THF (5 mL) and stirred at room temperature overnight. Following the similar workup procedure described for compound **6.19**, afforded **6.41** as a white solid (*E*-isomer - 450 mg and *E*+*Z* mixture - 240 mg, 82%). Data for *E*-isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.10 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $C(CH_3)_3$), 1.20 (s, 9H, Piv $C(CH_3)_3$), 2.21 - 2.42 (m, 2H, 2′-H), 4.25 - 4.38 (m, 2H, 3′ & 4′-H), 4.81 - 4.95 (m, 2H, 5-CH_2O), 5.72 (dd, *J* = 16.40, 1.76 Hz, 1H, 6′-H), 6.24 (t, *J* = 6.44 Hz, 1H, 1′-H), 6.79 (dd, *J* = 16.26, 4.54 Hz, 1H, 5′-H), 7.54 (s, 1H, 6-H), 9.34 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.84, -4.66 ($SiCH_3$), 17.89 ($C(CH_3)_3$), 25.63 ($C(CH_3)_3$), 27.12 ($C(CH_3)_3$), 38.91 (Piv $C(CH_3)_3$), 40.10 (2′-C), 58.36 (5-CH_2O-C), 74.53 (3′-C), 85.12 (4′-C), 86.07 (1′-C), 101.41 (6′-C), 110.38 (7′-C), 116.49 (5-C), 140.93 (6-C), 149.39 (5′-C), 149.70 (2-C), 162.32 (4-C), 178.84 (Piv CO). ESI-HRMS for $[C_{23}H_{35}N_3O_6Si + H]^+$ calcd, 478.2373; found, 478.2383.

(1-((2R,4S)-5-(2-cyanoethylidene)-4-hydroxytetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.42): To a solution of compound **6.41** (65 mg, 0.136 mmol) in THF (1 mL) was added tetrabutylammonium hydroxide ($Bu_4N^+OH^-$, 40% in H_2O, 186 mg, 0.286 mmol) and stirred at room temperature for 5h. Volatiles were evaporated under reduced pressure and the residue was purified by flash column chromatography (40-70% EtOAc in hexanes) to afford **6.42** as a white powder (20 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.19 (s, 9H, $C(CH_3)_3$), 2.20 (d, *J* = 3.51 Hz, 1H, 3′-OH), 2.31 (dt, *J* = 13.84, 6.70 Hz, 1H, 2′-H), 2.56 (ddd, *J* = 14.06, 6.44, 2.64 Hz, 1H, 2′-H), 3.17 (dd, *J* = 7.03, 1.17 Hz, 2H, 6′-H), 4.77 (t, *J* = 7.03 Hz, 1H, 5′-H), 4.83 - 4.92 (m, 3H, 3′-H & 5-CH_2O-H), 6.62 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.38 (s, 1H, 6-H), 8.46 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 13.70 (6-C), 27.10 ($C(CH_3)_3$) 38.90 ($C(CH_3)_3$), 39.22 (2′-C), 58.48 (5-CH_2O-C), 70.25 (3′-C), 87.13 (1′-C), 89.96 (5′-C), 111.06 (5-C), 117.77 (7′-C), 139.55 (6-C),

149.40 (2-C), 159.39 (4-C), 161.42 (4′-C), 178.68 (Piv CO). ESI-HRMS for $[C_{17}H_{21}N_3O_6 + H]^+$ calcd, 364.1509; found, 364.1455.

(1-((2R,4S,5R)-4-((*tert*-butyldimethylsilyl)oxy)-5-(2-cyanoethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.43): To a solution of **6.41** (240 mg, 0.5 mmol) in ethylacetate (10 mL) was added 5%Pt-C (250 mg) and stream of hydrogen gas was bubbled through the reaction mixture for 4h. The catalyst was filtered off and the filtrate was concentrated. The residue was purified by flash column chromatography (20-40% EtOAc in hexanes) to afford **6.43** as a white solid (170 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.09 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $C(CH_3)_3$), 1.20 (s, 9H, Piv $C(CH_3)_3$), 1.94 (dddd, *J* = 13.88, 9.63, 7.54, 6.15 Hz, 1H, 5′-H), 2.02 - 2.17 (m, 1H, 5′-H), 2.23 (ddd, *J* = 13.40, 7.25, 5.71 Hz, 1H, 2′-H), 2.34 (ddd, *J* = 13.77, 7.03, 5.86 Hz, 1H, 2′-H), 2.43 - 2.65 (m, 2H, 6′-H), 3.85 (ddd, *J* = 9.37, 5.42, 3.66 Hz, 1H, 4′-H), 4.16 (dt, *J* = 11.13, 5.57 Hz, 1H, 3′-H), 4.88 (s, 2H, 5-CH_2O), 6.12 (dd, *J* = 6.74, 5.86 Hz, 1H, 1′-H), 7.55 (s, 1H, 6′-H), 9.13 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.88, -4.61 ($SiCH_3$), 14.22 (6′-C), 17.86 ($C(CH_3)_3$), 25.64 ($C(CH_3)_3$), 27.09 ($C(CH_3)_3$), 28.96 (5′-C), 38.86 (Piv $C(CH_3)_3$), 40.48 (2′-C), 58.38 (5-CH_2O), 74.27 (3′-C), 84.26 (4′-C), 85.84 (1′-C), 109.99 (5-C), 118.95 (7′-C), 141.07 (6-C), 149.64 (2-C), 162.27 (4-C), 178.77 (Piv CO). ESI-HRMS for $[C_{23}H_{37}N_3O_6Si + H]^+$ calcd, 480.2530; found, 480.2535.

(1-((2R,4S,5R)-5-(2-cyanoethyl)-4-hydroxytetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.44): Following the synthetic procedure described for compound **6.20**, compound **6.43** (80 mg, 0.17 mmol) afforded product **6.44** as a white solid **(**55 mg, 90%). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.18 (s, 9H, $C(CH_3)_3$), 1.92 - 2.06 (m, 1H, 5′-H) 2.06 - 2.16 (m, 1H, 5′-H), 2.29 (dd, *J* = 6.59, 5.71 Hz, 2H, 2′-H), 2.52 - 2.71 (m, 2H, 6′-H), 3.87 (dt, *J* = 9.01, 4.43 Hz, 1H, 4′-H), 4.17 - 4.24 (m, 1H, 3′-H), 4.84 (s, 2H, 5-CH_2O), 6.20 (t, *J* = 6.59 Hz, 1H, 1′-H), 7.78 (s,1H, 6-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 14.51 (6′-C), 27.53 ($C(CH_3)_3$), 30.37(5′-C), 39.90 ($C(CH_3)_3$), 40.30 (2′-C), 60.22 (5-CH_2O), 74.70 (3′-C), 86.05 (4′-C), 86.85 (1′-C), 110.70 (5-C), 120.82 (7′-C), 143.01 (6-C), 151.87 (2-C), 164.85 (4-

C), 180.09 (Piv CO). ESI-HRMS for $[C_{17}H_{23}N_3O_6 + H]^+$ calcd, 366.1665; found, 366.1631.

3-((2R,3S,5R)-3-hydroxy-5-(5-(hydroxymethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)propanenitrile (6.30): A solution of 0.5M NaOMe in MeOH (0.7 mL, 0.342 mmol) was added to compound **6.44** (25 mg, 0.068 mmol) and stirred at room temperature for 3h. Acetic acid (30 µL, 0.5 mmol) was added and the solvents were evaporated. The residue was purified by flash column chromatography (4-8% MeOH in CH_2Cl_2) to afford **6.30** as a white solid (19 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.76 - 1.90 (m, 1H, 5′-H), 1.90 - 2.03 (m, 1H, 5′-H), 2.06 - 2.25 (m, 2H, 2′-H), 2.53 - 2.68 (m, 2H, 6′-H), 3.72 (dt, *J* = 8.49, 4.54 Hz, 1H, 4′-H), 4.05 - 4.13 (m, 1H, 3′-H), 4.16 (s, 2H, 5-CH_2OH), 4.97 (br. s, 1H, 5-CH_2O*H*), 5.39 (br. s, 1H, 3′-OH), 6.16 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.42 (s, 1H, 6-H), 11.34 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 13.21 (6′-C), 28.53 (5′-C), 38.36 (2′-C), 55.70 (5-CH_2OH), 72.53 (3′-C), 83.87 (1′-C), 83.97 (4′-C), 114.46 (5-C), 120.17 (7′-C), 136.38 (6-C), 150.13 (2-C), 162.42 (4-C). ESI-HRMS for $[C_{12}H_{15}N_3O_5 + HCOO^-]^-$ calcd, 326.0988; found, 326.0985.

(1-((2R,4S,5R)-5-(2-(1H-tetrazol-5-yl)ethyl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl pivalate (6.45): Following the procedure described for the synthesis of **6.21**, compound **6.43** (80 mg, 0.17 mmol) rendered **6.45** as white solid (74 mg, 74 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08 (s, 6H, $SiCH_3$), 0.89 (s, 9H, $C(CH_3)_3$), 1.24 (s, 9H, Piv $C(CH_3)_3$), 1.74 - 1.91 (m, 1H, 5′-H), 2.04 - 2.17 (m, 1H, 2′-H), 2.19 - 2.33 (m, 1H, 5′-H), 2.39 (ddd, *J* = 13.55, 5.93, 3.95 Hz, 1H, 2′-H), 3.13 - 3.28 (m, 2H, 6′-H), 4.03 (dt, *J* = 10.69, 3.00 Hz, 1H, 4′-H), 4.10 - 4.18 (m, 1H, 3′-H), 4.93 - 5.08 (m, 2H, 5-CH_2O), 6.21 (t, *J* = 6.30 Hz, 1H, 1′-H), 7.79 (s, 1H, 6-H), 8.99 (br. s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm -4.83, -4.66 ($SiCH_3$), 17.95 ($C(CH_3)_3$), 20.40 (6′-C), 25.68 ($C(CH_3)_3$), 27.12 ($C(CH_3)_3$), 33.34 (5′-C), 39.38 (Piv $C(CH_3)_3$), 40.56 (2′-C), 59.14 (5-CH_2O), 75.13 (3′-C), 86.66 (4′-C), 87.00 (1′-C), 109.27 (5-C), 142.20 (6-C), 149.73 (2-C), 155.28 (7′-C), 162.27 (4-C), 182.48 (Piv CO). ESI-HRMS for $[C_{23}H_{38}N_6O_6Si - H]^-$ calcd, 521.2544; found, 521.2551.

1-((2R,4S,5R)-5-(2-(1H-tetrazol-5-yl)ethyl)-4-((*tert*-butyldimethylsilyl)oxy) tetrahydrofuran-2-yl)-5-(hydroxymethyl)pyrimidine-2,4(1H,3H)-dione (6.46): Following the procedure described for the synthesis of **6.30**, compound **6.45** (90 mg, 0.17 mmol) after chromatography (5-7 % MeOH + 0.5 % HCOOH in CH_2Cl_2) rendered **6.46** as white solid (70 mg, 92 %). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.11 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 2.00 - 2.12 (m, 1H, 5′-H), 2.12 - 2.38 (m, 3H, 2′ & 5′-H), 2.99 - 3.19 (m, 2H, 6′-H), 3.82 (dt, *J* = 9.37, 4.10 Hz, 1H, 4′-H), 4.31 (dt, *J* = 6.00, 4.32 Hz, 1H, 3′-H), 4.35 (d, *J* = 1.17 Hz, 2H, 5-CH_2OH), 6.20 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.53 (s, 1H, 6-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm -4.70, -4.50 ($SiCH_3$), 18.80 ($C(CH_3)_3$), 21.38 (6′-C), 26.25 ($C(CH_3)_3$), 32.52 (5′-C), 40.87 (2′-C), 57.80 (5-CH_2OH), 76.34 (3′-C), 86.61 (1′-C), 87.00 (4′-C), 115.70 (5-C), 138.70 (6-C), 152.12 (2-C), 158.26 (7′-C), 165.00 (4-C). ESI-HRMS for $[C_{18}H_{30}N_6O_5Si - H]^-$ calcd, 437.1969; found, 437.1982.

1-((2R,4S,5R)-5-(2-(1H-tetrazol-5-yl)ethyl)-4-hydroxytetrahydrofuran-2-yl)-5-(hydroxymethyl)pyrimidine-2,4(1H,3H)-dione (6.47): In a polypropylene vessel, to a solution of compound **6.46** (70 mg, 0.16 mmol) in MeOH (10 mL) was added NH_4F (120 mg, 3.2 mmol) and stirred at 50 °C for 2 days. CH_2Cl_2 (10 mL) was added and filtered. The filtrate was concentrated and the residue purified by flash column chromatography (8-12 % MeOH + 0.5% HCOOH in CH_2Cl_2) to afford **6.47** as a white solid (45 mg, 87%).^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.88 - 2.01 (m, 1H, 5′-H), 2.01 - 2.24 (m, 3H, 5′ & 2′-H), 2.86 - 3.05 (m, 2H, 6′-H), 3.65 - 3.75 (m, 1H, 4′-H), 4.05 - 4.13 (m, 1H, 3′-H), 4.16 (d, *J* = 0.88 Hz, 2H, 5-CH_2OH), 6.15 (t, *J* = 6.74 Hz, 1H, 1′-H), 7.44 (s, 1H, 6-H), 11.35 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.65 (6′-C), 30.89 (5′-C), 38.63 (2′-C), 55.69 (5-CH_2OH), 72.77 (3′-C), 83.60 (1′-C), 84.50 (4′-C), 114.48 (5-C), 136.18 (6-C), 150.14 (2-C), 155.96 (7′-C), 162.42 (4-C). ESI-HRMS for $[C_{12}H_{16}N_6O_5 - H]^-$ calcd, 323.1104; found, 323.1234.

6.4.2. Pharmacological assay procedures

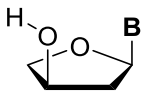
Please refer to Chapter 3, Section 3.4.2.5 for spectrophotometric binding assay procedure

CHAPTER – 7

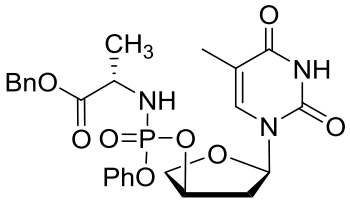
GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

General Conclusions

A series of previously undisclosed 2′-deoxy-α-L-threofuranosylnucleosides (**3.25**-**27** and **3.29**), synthesized from 1,2-*O*-isopropylidene-α-L-threofuranose in seven steps, failed to show antiviral activity. Assuming that the incapability of cellular kinases to execute the first phosphorylation step (unprecedented on a secondary hydroxyl of a nucleoside substrate) was responsible for the lack of activity, we decided to synthesize prodrug **3.34**, which, however, also lacked antiviral activity. Hindering factors may be the inefficient processing to the corresponding monophosphate (although we demonstrated fluent conversion to the alanylmonophosphate precursor upon carboxypeptidase treatment of the thymine analogue), inefficient conversion of the mono- to the triphosphate form or the incapacity of the triphosphate to act as RT substrate/inhibitor.



3.25: B = T
3.26: B = U
3.27: B = A
3.29: B = C

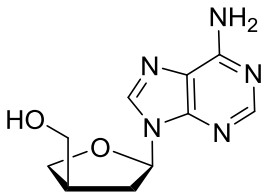


3.34

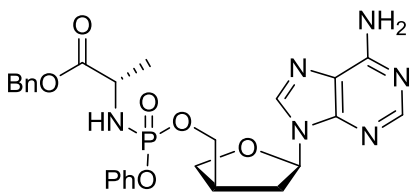
Therefore, we decided to revisit the corresponding 2′,3′-dideoxy-β-D-apio-D-furanose nucleosides, which may be considered as homologues of the aforementioned 2′-deoxy-α-L-threofuranosylnucleosides. We envisaged a synthetic approach that would also give access to the corresponding 3′-deoxy-D-apio-D-furano nucleosides and D-apio-D-furanonucleosides (Chapter 4). In the course of our attempts to synthesize this family of apionucleosides, we discovered anomalies in the structural assignments of related compounds reported by Jin *et al*. and also experienced that a compound purchased from the chemical supplier Carbosynth Ltd as 1,2:3,5-di-*O*-isopropylidene-α-D-apio-D-furanose proved to be the D-apio-L-furanose epimer, which demonstrates the importance of using a nomenclature that unambiguously denotes the stereochemistry at carbons 2 and 3 of this unusual sugar. Clues in the synthesis of the

desired apionucleosides were a single carbon homologation, a tandem acid catalyzed ring opening followed by differential ring closing and an optimized microwave assisted glycosylation protocol.

In accordance with earlier reports the target D-apio-D-furanose nucleosides failed to show antiviral activity and so did their D-apio-L-furanose epimers. However, the triphosphate of 2′,3′-dideoxy-β-D-apio-D-furanoadenosine (**4.1b**) (in contrast to its D-apio-L-furanose epimer) was readily accepted by viral DNA polymerase to act as chain terminator. This led us to convert **4.1b** into the phosphoramidate prodrug **4.6b**, which indeed showed considerable anti-HIV activity. This indicates that the lack of activity of these 2′,3′-dideoxy-β-D-apio-D-furanose nucleosides must be sought in the inefficient conversion to the monophosphate.

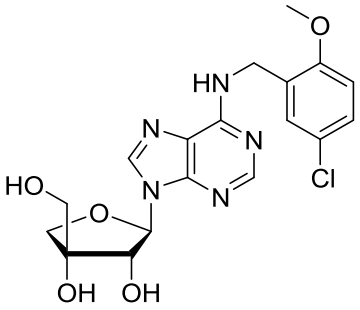


4.1b

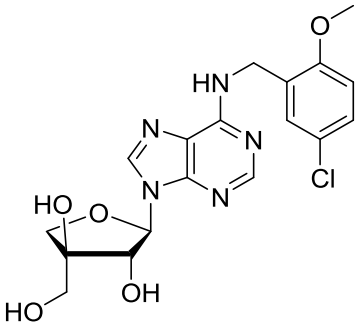


4.6b
EC_{50} = 0.5 - 1.5 μM
against HIV - 1,2

The synthetic methodology developed to synthesize the family of apionucleosides was applied for the preparation of N^6-aralkyl-β-D-apio-D/L-furanoadenosines, as well as their 5′-carboxamide or ethyl/methyl carbamoyl derivatives (Chapter 5). Some analogues showed moderate affinity for adenosine A_3 receptors. Surprisingly, D-apio-D-furanoadenosine analogue **5.26** behaved as an antagonist for this receptor, while its D-apio-L-furanoadenosine counterpart **5.7** showed partial agonist activities. This stereochemical preference to activate this target receptor is in striking contrast with that earlier observed for HIV RT. The relatively low affinity of these apionucleosides (compared to the natural ribonucleosides) for this purinergic receptor may be explained by the fact that they adopt an inapt conformation for receptor recognition.

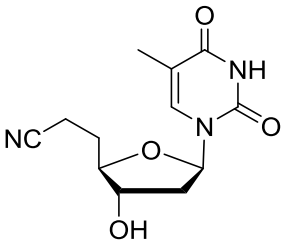


5.26: Antagonist
K_i = 0.98 μM

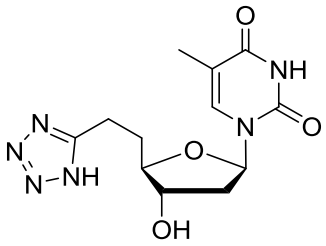


5.7: Agonist
K_i = 3 μM

Selected 5′-modified thymidine congeners are moderate inhibitors of TMPK*mt* (Chapter 6). Unfortunately, combining the most favorable 5′-modification (**6.20**, **6.22**) with a 5-CH_2OH was detrimental for inhibitory activity, which may indicate the QSAR from which this combination emerged, is only valuable in the presence of a fused 2′,3′-sulfuryldiamide ring. The protecting group strategy employed for the 5-CH_2OH group may prove practical for the synthesis of analogues of 5-hydroxymethyl-2′-deoxycytidine, believed to have an important role in embryo development and in the epigenetic control of neuronal function.



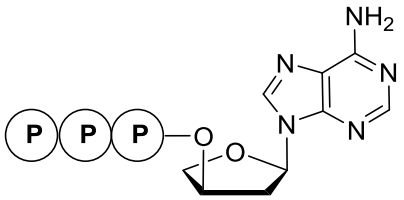
6.20: K_i = 48 μM



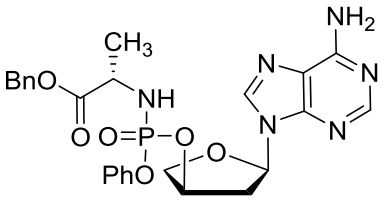
6.22: K_i = 70 μM

Future Perspectives

In general, inhibition of HIV viruses by thymine nucleoside analogues is poor compared to their adenine counterparts. In the case of 2′,3′-dideoxy-β-D-apio-D-furanose nucleosides, the prodrugs of adenine analogues were found to have potent anti-HIV properties (**4.6b** and **4.80b**), while their thymine congeners were only weakly active. Hence, it would be interesting to investigate whether the triphosphate of 2′-deoxythreoadenosine (**7.1**) could terminate growing DNA/ inhibit HIV RT enzyme. If affirmative, the synthesis and screening of **7.2** may be rewarding.



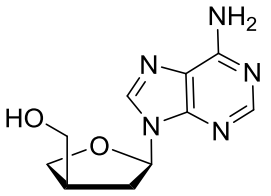
7.1



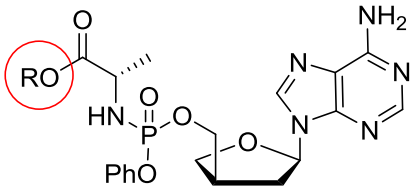
7.2

As exemplified for 2′,3′-dideoxy-β-D-apio-D-furanoadenosine (**4.1b**) the inactivity of many nucleoside analogues is largely due to inefficient phosphorylation(s). testing final metabolites (triphosphates in these cases) is perhaps more prudent. To uncover promising RT or polymerase inhibitors it might therefore be worthwhile to systematically screen the triphosphate metabolites of nucleosides that were previously found inactive. Ready availability of these data could create opportunities to convert (old) nucleosides analogues to promising (pro)drugs.

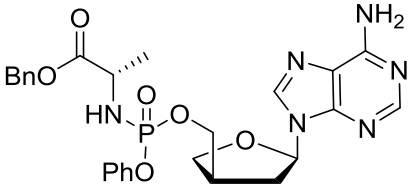
Obviously, the antiviral activity of ProTides is critically dependent on the ester type of the amino acid. The benzyl ester **4.6b** for instance is at least 15 times more active than the isopropyl ester in vitro, against HIV 1,2. Therefore, a more systematic screening of a library of ProTides **7.3** with various ‘R’ groups is warranted.



4.1b

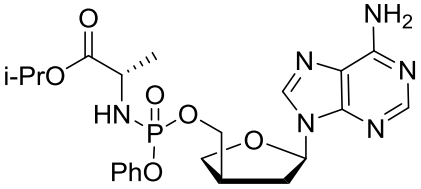


7.3



4.6b

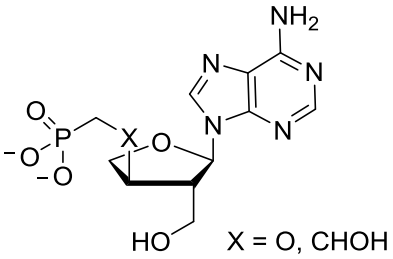
EC_{50} = 0.5 - 1.5 μM



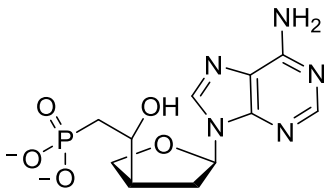
4.80b

EC_{50} = 7.5 - 38 μM

Oxetanocin and their ring expanded analogues are potent antiviral compounds. Still, synthesis and activity of their phosphonate equivalents **7.4** are not reported. Similarly, the hydroxyl compound **7.5** and α-difluorophosphonate analogues of **7.4** and **7.5** are worth investigation.

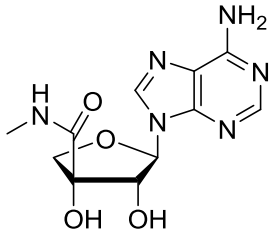


7.4

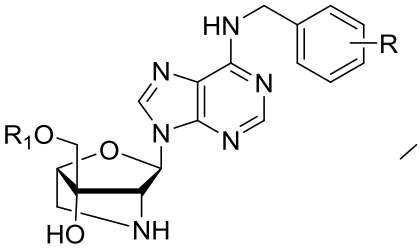


7.5

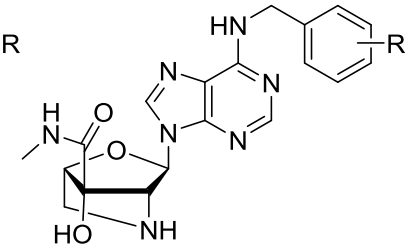
Based on the observation that the oxidation of 3′-CH_2OH of the α-D-apio-L-furanoadenosine to carboxylic acid resulted in decarboxylation, synthesis of 3′-carboxamide **7.6** as adenosine A_3 receptor modulator was not attempted, but access to **7.6** may be feasible using a different protecting/ synthetic strategies like, a 2′,3′-*O*-isopropylidene group followed by a screen of various oxidation methods.



7.6



7.7: R_1 = H
7.8: R_1 = CONHMe



7.9

We attributed the inactivity of the D-apio-L-furano and D-furanoadenosines to its inapt conformations, moreover, 2′,4′-locked adenosines are reported as potent antagonists, hence efforts to determine affinity profiles of the north-locked congeners **7.7**-**7.9** may be rewarding.

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SUMMARY

Nucleoside analogues with different modes of action have proven useful for treating various diseases, particularly for viral infections. To act as antivirals nucleosides generally require metabolic activation to their triphosphate form by nucleoside kinases. The triphosphates then act as inhibitors of viral DNA or RNA polymerases or as DNA or RNA chain terminators. Nucleos(t)ide prodrugs have been developed to increase bioavailability or to bypass the first phosphorylation step.

In Chapter 1 we tried to give the relevant chemical and biological background for the research work performed in this thesis, *i.e*. nucleosides as antivirals, A_3 adenosine receptor ligands and *M. tuberculosis* TMPK inhibitors. We provide quite an elaborate overview of nucleosides in which either the base or the 5′-CH_2OH has been transposed and the effects of such modifications on the biological activity.

Chapter 2 summarizes the goals of this thesis.

In Chapter 3 and 4 of this thesis we describe the synthesis of sugar-modified nucleosides as potential antivirals. Chapter 3 is dedicated to the synthesis of a series of α-L-2′-deoxythreofuranosyl nucleosides featuring the nucleobases A, T, C and U. The target molecules were synthesized from 1,2-*O*-isopropylidene-α-L-threose, involving a Vorbrüggen coupling and a Barton-McCombie deoxygenation protocol as the key steps. All target nucleosides failed to show significant activity against a broad panel of viruses and also lacked notable cellular toxicity. Therefore, we decided to convert the thymidine analogue to a phosphoramidate prodrug, which, however, failed to restore the antiviral activity. Evaluation against a number of thymidine kinases learned that 1′-(thymin-1-yl)-2′-deoxy-α-L-threofuranose causes moderate inhibition of mitochondrial thymidine kinase-2 (TK-2; IC_{50}: 429 µM), herpes simplex virus type 1 TK (IC_{50}: 66 µM, K_i: 20 µM), varicella-zoster virus TK (IC_{50}: 123 µM) and *M. tuberculosis* thymidylate kinase (K_i: 18 µM).

In Chapter 4 we describe a new and convenient synthesis towards thymine and adenine congener of D-apio- L- and D-furanonucleosides, their 3′-deoxy-, as well as

their 2′,3′-dideoxy-analogues. In the course of this work, we rectified some anomalies in the structure assignments reported by other groups. Single carbon homologation of the carbohydrate moiety and optimized glycosylation conditions involving microwave irradiation allowed the successful synthesis of the target compounds.

Initially, following a reported method we arrived at a family of unintended D-apio-L-furanonucleosides. These nucleosides and prodrugs of their 2′,3′-dideoxy counterparts were devoid of antiviral activity. The 1′-(Thymin-1-yl)-2′,3′-dideoxy-α-D-apio-L-furanose was poorly active as inhibitor of several viral thymidine kinases (IC_{50}s in the 150-270 μM range), moderately active as inhibitor of thymidylate kinase of *M. tuberculosis* (K_i: 48 μM) and the triphosphate of 1′-(adenin-9-yl)-2′,3′-dideoxy-α-D-apio-L-furanose proved a poor substrate of HIV RT. After overcoming some synthetic challenges, a group of β-D-apio-D-furano target nucleosides and relevant ProTides were prepared. Although the nucleosides themselves are neither active nor cytotoxic, the ProTides of 1′-(adenin-9-yl)-2′,3′-dideoxy-β-D-apio-D-furanose are moderately potent anti-HIV-1,2 (EC_{50}: 0.5-38 μM) agents, showing weak cytotoxicity (IC_{50}: 53-110 μM). Accordingly, 2′,3′-dideoxy-β-D-apio-D-furanoadenosine triphosphate is readily accepted by HIV RT and acts as viral DNA chain terminator.

Adenosine receptors are GPCRs that are activated by extracellular adenosine to modulate signal transduction pathways inside the cell. Selective A_3 adenosine receptor modulators are being investigated in the clinic for controlling inflammatory disorders (rheumetoid arthritis), immune diseases (psoriasis), dry eye syndrome, glaucoma and colorectal cancer. In search of new modulators of this receptor, we synthesized a small series of N^6-benzyl substituted 9-(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenosines (Chapter 5). Because the starting material supplied had the undesired stereochemistry at C-3 of apiofuranose (see also Chapter 4), early work in this study resulted in 9-(3-*C*-hydroxymethyl-α-L-threofuranosyl)adenosines and its 3′-deoxy congeners. After evaluation of all compounds, N^6-(5-chloro-2-methoxybenzyl) derivative of D-apio- L- and D-furanoadenosines showed appreciable binding affinity to the A_3AR, but with affinity 100-fold lower than the parent N^6-(5-chloro-2-methoxy)benzyladenosine. All together it may be concluded that substitution

of the apiofuranose for a ribofuranose moiety is detrimental for binding to the adenosine receptors.

TMPK of *M. tuberculosis* is responsible for converting deoxythymidine monophophate to deoxythymidine diphosphate, a crucial step in the biosynthesis of bacterial DNA. Since TMPK*mt* lies at the junction of *de novo* and *salvage* pathways for DNA biosynthesis and significantly differs from its human counterpart, it is deemed an attractive target for designing new TB drugs. In Chapter 6 we describe the synthesis of 5′-modified thymidines and 5,5′-bis-substituted 2′-deoxyuridine analogues as potential inhibitors of TMPK*mt*. A lot of efforts were made to find a protecting group for the 5-CH_2OH moiety of 2′-deoxyuridines, that enables to introduce the desired 5′-modifications. The 5′ and 5,5′-modified analogues were evaluated for their capacity to inhibit TMPK*mt*. Solely the 5′-cyanomethyl (K_i: 48 µM) and 5′-tetrazolylmethyl (K_i: 70 µM) modified thymidines were found to possess moderate TMPK*mt* inhibitory activity. Remarkably, compounds in which these favorable 5′-modifications were combined with a 5-CH_2OH modification of the nucleobase, failed to inhibit the enzyme.

Samenvatting in het Nederlands

Nucleoside analogen met uiteenlopende werkingsmechanismen hebben bewezen buitengewoon effectief te zijn bij de behandeling van een scala aan ziekten, in het bijzonder virale infecties. Voor het verkrijgen van deze antivirale activiteit, dienen nucleosiden, in de regel, door middel van nucleoside kinasen te worden omgezet in hun corresponderende trifosfaat vorm. Deze trifosfaten kunnen vervolgens optreden als inhibitor van virale DNA- of RNA-polymerasen en/of als DNA- of RNA-keten terminatoren.

In Hoofdstuk 1 is ernaar gestreefd een relevant chemische en biologische achtergrond te schetsen voor het werk uiteengezet in dit proefschrift, te weten: antivirale nucleosiden, A_3 adenosine receptor liganden en inhibitoren van TMPK in *M. tuberculosis*. Wij bieden een gedetailleerd overzicht van tal van nucleosiden waarin de base en/of de 5′-CH_2OH is verplaatst en het effect hiervan op de biologische activiteit.

In Hoofdstuk 2 staan de doelstellingen van dit proefschrift uiteengezet.

De Hoofdstukken 3 en 4 beschrijven de synthese van suiker-gemodificeerde nucleosiden als mogelijk antivirale agentia. Hoofdstuk 3 beschrijft de synthese van een reeks α-L-2′-desoxythreofuranosylnucleosiden uitgerust met de nucleobasen A, T, C and U. De doelmoleculen werden verkregen uit 1,2-*O*-isopropylideen-α-L-threose, met, onder meer, een Vorbrüggen koppeling en een Barton-McCombie deoxygenering als voornaamste omzettingen. Geen van de verkregen nucleosiden vertoonde een significante activiteit tegen een breed scala aan virussen. De nucleosiden vertoonden bovendien een (zeer) lage celtoxiciteit. Dit zette er ons toe aan het thymidine analoog om te zetten in een fosforamidaat prodrug, wat echter geen aanleiding gaf tot een verhoogde antivirale activiteit.

Enzym inhibitiestudies toonden aan het 1′-(thymin-1-yl)-2′-deoxy-α-L-threofuranose een zwakke inhibitor is van: mitochondriaal thymidine kinase-2 (TK-2; IC_{50}: 429 µM), herpes simplex virus type 1 TK (IC_{50}: 66 µM, K_i: 20 µM), varicella-zoster virus TK (IC_{50}: 123 µM) en thymidylaat kinase van *M. tuberculosis* (K_i: 18 µM).

Hoofdstuk 4 beschrijft een nieuwe en efficiënte synthese van zowel de D- als de D-apio-L-furanonucleosiden van thymine en adenine, alsook hun corresponderende 3′-deoxy-, en 2′,3′-dideoxyanalogen. Gedurende het verloop van dit onderzoek werd een onjuistheid in de stereochemische toekenning van een eerder door een andere onderzoeksgroep beschreven, intermediar vastgesteld en gecorrigeerd. Homologatie van het glycon met één koolstofatoom en glycosylering in een microgolfoven speelden een sleutelrol in de synthese van deze derivaten.

Aanvankelijk werd gebaseerd op een gepubliceerde methode een reeks van (onbedoelde) D-apio-L-furanonucleosiden verkregen. Deze nucleosiden en de prodrugs van hun respectieve 2′,3′-dideoxy analogen vertoonden geen noemenswaardige antivirale activiteit. 1′-(Thymin-1-yl)-2′,3′-dideoxy-β-D-apiose blijkt een zwakke inhibitor te zijn van virale thymidine kinasen (IC_{50}s circa 150-270 μM) en een matig potente inhibitor van thymidylaat kinase van *M. tuberculosis* (K_i: 48 μM). Het trifosfaat van 1′-(adenin-9-yl)-2′,3′-dideoxy-α-D-apio-L-furanose bleek een pover substraat van HIV RT. Na het nemen van enkele synthetische horden werd de initieel voorgenomen groep D-apio-D-furanonucleosiden (inclusief de relevante ProTides) verkregen. Hoewel de nucleosiden op zichzelf actief noch cytotoxisch zijn, blijken de ProTides van 1′-(adenin-9-yl)-2′,3′-dideoxy-β-D-apio-D-furanose relatief potente anti-HIV-1,2 (EC_{50}: 0.5-38 μM) agentia, met een lage cytotoxiciteit (IC_{50}: 53-110 μM). Overeenkomstig blijkt het corresponderende 2′,3′-dideoxy-β-D-apio-D-furanoadenosine trifosfaat een goed substraat van HIV RT en treedt daarbij op als virale DNA ketenterminator.

Adenosine receptoren behoren tot de GPCRs en zijn, na activatie door extracellulair adenosine, betrokken bij signaaltransductie in de cel. De waarde van selectieve A_3 adenosine receptor modulatoren in de behandeling van ontstekingen (reumatoïde artritis), immuunziekten (psoriasis), droge ogen syndroom, glaucoom en darmkanker wordt thans klinisch onderzocht.

In de zoektocht naar nieuwe modulatoren van deze receptor werd een kleine reeks N^6-gesubstitueerde 9-(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adeninen gesynthetiseerd (Hoofdstuk 5). Aangezien bij aanvang van deze studie werd

vertrokken van een startmateriaal met de verkeerde configuratie op C-3 van apiofuranose (zie Hoofdstuk 4), werden initieel 9-(3-*C*-hydroxymethyl-α-L-threofuranosyl)adenosinen en hun 3′-deoxy analogen verkregen.

Bij de biologische evaluatie van alle verbindingen bleken de N^6-(5-chloro-2-methoxybenzyl) derivaten van D-apio-L- en D-furanoadenosine een behoorlijke bindingsaffiniteit te vertonen voor de A_3 adenosine receptor, maar toch een factor 100 lager dan die van de oorspronkelijke verbinding N^6-(5-chloro-2-methoxy)benzyladenosine. Dit alles in ogenschouw nemend, kan worden geconcludeerd dat het vervangen van een ribofuranose door een apiofuranose structuur nadelig is voor de binding aan de adenosine receptor.

TMPK van *M. tuberculosis* katalyseert de omzetting van deoxythymidinemonofosfaat in deoxythymidinedifosfaat, een sleutelstap in de bacteriële biosynthese van DNA. TMPKmt wordt beschouwd als een aantrekkelijk aangrijpingspunt voor het ontwerp van nieuwe TB medicatie aangezien het werkzaam is op het punt waar de *de novo* en *salvage* paden voor DNA biosynthese samenvloeien en het bovendien significant verschillend is van het analoge enzym in de mens.

Hoofdstuk 6 behandelt de synthese van 5′-gemodificeerde thymidinen en 5,5′-bisgesubstitueerde 2′-deoxyuridine analogen. Veel aandacht ging hierbij uit naar de zoektocht naar een geschikte beschermgroep voor de 5-CH_2OH groep van 2′-deoxyuridinen die verenigbaar is met de transformaties noodzakelijk voor de invoering van de 5′-modificaties. De 5′ en 5,5′-gemodificeerde analogen werden getest op de inhibitie van TMPK*mt*. Het 5′-cyanomethylderivaat (K_i: 48 μM) en het 5′-tetrazolylmethylderivaat (K_i: 70 μM) van thymidine vertoonden bescheiden TMPKmt inhibitie. Vreemd genoeg vertoonden de verbindingen waarin deze 5′-modificaties werden gecombineerd met een 5-CH_2OH groep op de nucleobase, geen significante remming van TMPK*mt*.

SCIENTIFIC CV

Personal Details:

Name, surname:	Kiran Shambhu, Toti
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Educational Qualifications:

- ***PhD student*** (March 2009 – till date) at Laboratory of Medicinal Chemistry, University of Gent, Belgium (Supervisor: Prof. Dr. Serge Van Calenbergh).
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- ***Master of Science*** (Organic Chemistry), June 2004, First class with Distinction (75.87%), Karnatak University, Dharwad, India.
- ***Bachelor of Science*** (Chemistry, Physics and Mathematics), May 2002, First class with Distinction (70%), JSS College, Karnatak University, Dharwad, India.

Past Research Career:

- ***Research Associate*** **(**July, 2007 - January 2009) at Aurigene Discovery Technologies, Bangalore, India (subsidiary of Dr. Reddy's Laboratories).
- ***Project Assistant*** (August, 2004 - July, 2007) at Division of Organic Chemistry: Technology, National Chemical Laboratory (NCL) Pune, India.

Distinctions:

- Qualified ***National Eligibility Test for Lectureship*** (CSIR-UGC NET) in Chemical Sciences conducted jointly by Council of Scientific and Industrial Research (CSIR) and University Grants Commission (UGC) New Delhi, India held in June 2004.

- Stood ***merit second to university*** in Master of Science (Organic Chemistry) Examination conducted by Karnatak University for the year 2002-2004.

Publications and patent:

[1] Nucleobase or 4′-hydroxymethyl transposed nucleosides and nucleotides: synthesis and biological activity. Kiran Toti, Elisabetta Groaz, Marleen Renders, Piet Herdewijn and Serge Van Calenbergh* *A review in preparation*.

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Supervision of master students:

- Frederick Verbeke
  Thesis title: Synthesis of *in silico* suggested inhibitors of Thymidylate Kinase of *Mycobacterium Tuberculosis*, 2011
- Arpit Bhandari
  Summer internship: Synthesis of modified sugars, 2011
- Sunil Kumar Kataria
  Summer internship: Synthesis of antiviral nucleoside phosphonates, 2012

Conferences and Courses:

12th Chemistry Conference for Young Scientists
Blankenberge, Belgium, February 27-28, 2014
Oral Presentation: Synthesis and biological evaluation of 4′-Hydroxymethyl Deleted, Transposed and Modified Nucleosides

XX International roundtable on Nucleosides Nucleotides and Nucleic acids
Montreal, Canada, August 5-9, 2012
(*Received Travel Award from Conference Organisers*)
Poster Presentation: Syntheses and biological evaluation of apionucleosides and their ProTides
Poster Presentation: 5,5'-bis substituted 2'-deoxyuridines as possible *M. tuberculosis* thymydylate kinase inhibitors

MedChem 2011 - Emerging Targets and Treatments (SRC & KVCV)
Ghent, Belgium, November 25, 2011

XV Symposium on Chemistry of Nucleic Acid Components

Cesky, Krumlov, Czech Republic, June 5-10, 2011

Poster Presentation: Dideoxyapiose nucleosides revisited: synthesis and ProTide derivatives

21st International Symposium on Medicinal Chemistry (EFMC-ISMC 2010)

Brussels, Belgium, September 5-9, 2010

Poster Presentation: L-2-deoxythreose nucleosides: synthesis and screening against viral strains

10th Flemish Youth Conference of Chemistry

Blankenberge, Belgium, March 1-2, 2010

Poster Presentation: Synthesis of L-2-deoxythreose nucleosides as potential antiviral agents

13th Sigma Aldrich Organic Synthesis Meeting

Spa, Belgium, December 3-4, 2009

Poster Presentation: Synthesis of L-2-deoxythreose nucleosides as potential antiviral agents

MedChem 2009 - Does size matter (SRC & KVCV)

Brussels, Belgium, November 2, 2009

18th School on Medicinal Chemistry (LACDR)

Oegstgeest, the Netherlands, October 27-30, 2009

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